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Leafy spurge (*Euphorbia* spp. near *esula*) Variability and its Effects Upon
Aphthona nigricutis (Coleoptera: Chrysomelidae) Feeding Preferences and
Congregation Behaviour

by

James Allen Tansey



A thesis submitted to the Faculty of Graduate Studies and Research in partial
fulfillment of the requirements for the degree of Master of Science

in

Environmental Biology and Ecology

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Faculty of Graduate Studies and Research

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled Leafy spurge (*Euphorbia* spp. near *esula*) Variability and its Effects Upon *Aphthona nigriscutis* (Coleoptera: Chrysomelidae) Feeding Preferences and Congregation Behaviour submitted by James Allen Tansey in partial fulfillment of the requirements for the degree of Master of Science in Environmental Biology and Ecology.

Abstract

Aphthona nigriscutis have established upon 69.5% of release sites in Alberta. Host variability may influence establishment. Feeding responses of *A. nigriscutis* to leafy spurge collected from release sites in Alberta indicated within- and among-site differences. A correlation between the variability of host plants and beetle establishment was evident. Because an aggregated distribution may allow these beetles to find mates, overcome host defenses and avoid predators, factors such as within-site host variability that contribute to it by influencing the attraction of *A. nigriscutis* to some plants may influence establishment.

A. nigriscutis are attracted to moderate densities of conspecifics and repelled by high densities. Male *A. nigriscutis* produced an aggregation pheromone and both females and males produced a dispersal pheromone at high densities. Attractive cues were secreted in the frass of male *A. nigriscutis* and both males and females produced repellent cues in another manner. The attraction of *A. nigriscutis* to conspecifics upon leafy spurge plants was influenced by variable host factors.

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Abbreviations

m	Metre
cm	Centimetre
mm	Millimetre
L	Litre
ml	Millilitre
in	Inch, Inches
\bar{x}	Sample Mean
s	Sample Standard Deviation
m	Population Mean
m^*	Lloyd's Mean Crowding Coefficient
σ^2	Population Variance
$^{\circ}\text{C}$	Degrees Celsius
$^{\circ}$	Degree
$\sqrt{}$	Square Root
\pm	Plus or Minus
hr	Hour, Hours
MST	Mountain Standard Time
TM	Trade Mark
ANOVA	Analysis of Variance
SNK	Student-Newman-Keul's

Chapter 1

Introduction

Leafy spurge, *Euphorbia* spp. near *esula* L. (Euphorbiaceae) is a deep-rooted, herbaceous perennial weed of Eurasian origin. Leafy spurge thrives in a number of habitats, ranging from sub-alpine to semi-aquatic, xeric to wet (Hanson and Rudd 1933) and in a number of soil types. Selleck et al. (1962) reported that in Saskatchewan 48% of infested quarters were in light loam to sand, 42% were in loam to clay loam and 10% were in clay to heavy clay soils.

Economically, leafy spurge is of greatest importance in areas used as rangeland. This is as much a management as a habitat classification and loosely describes any natural area upon which animals, including livestock, graze. These areas can be further characterized by an extensive as opposed to intensive management strategy that includes a minimum amount of deliberate manipulation and generally a polycultural plant community (Edward Bork *pers. comm.*).

Infestations have become a serious problem on the Great Plains of the United States and the Canadian Prairie Provinces. Economic losses exceeding \$120 million have been attributed to competition of leafy spurge with economically important forage species and the avoidance by cattle of infested areas of southern Canada and the northern United States (Leitch et al. 1994).

Leafy spurge. Competitiveness.

Belcher and Wilson (1989) demonstrated that the species richness and diversity of mixed-grass prairie and cover values of all common native plant species were negatively correlated to spurge infestation. Leafy spurge is very

competitive and can decrease herbage production in infested areas by 75% (Alley et al. 1984) to 100% (Gassman et al. 1996). These plants are often the first to emerge in the spring and therefore may gain an advantage over other plant species. Plants can reach a height of 15 cm by the first week in May in Saskatchewan (Messersmith et al. 1985). Competition for water, nutrients and light also favours leafy spurge because of its extensive root system, dense shoot growth and thick canopy (Hansen et al. 1997). However, as the greatest rate of spread occurs in more humid areas (Messersmith et al. 1985), the competitiveness and spread of leafy spurge is likely limited by moisture. The spread of leafy spurge can also be hindered, at least temporarily, by competition with some grass species. Morrow (1979) indicated that early emerging crops such as crested wheatgrass (*Agropyron desertorum*) or smooth brome (*Bromus inermis*) sod that monopolize moisture early in the season could limit the spread of infestations.

Leafy spurge. Allelopathy.

Leafy spurge is an allelopathic species; compounds exuded by these plants have an inhibitory effect on growth of other plant species. Few forbs grow within leafy spurge patches, even in bare spots between shoots (Steenhager and Zimdahl 1979). Aqueous extracts of leafy spurge shoot material suppressed the growth of pea (*Pisum sativum* L.) and wheat (*Triticum aestivum* L.) seedlings (Letourneau and Heggeness 1957). Field-collected soil samples from areas with moderate leafy spurge population inhibited the growth of potted tomatoes (*Lycopersicon esculentum* Mill.) in the greenhouse and dried plant material mixed with fresh soil

inhibited the growth of tomatoes and large crabgrass (*Digitaria sanguinalis* L.) (Steenhager and Zimdahl 1979). Addition of dried material to soil produced an inhibitory effect suggesting that the decomposition of dead leafy spurge may contribute to the allelopathic effect.

Leafy spurge. Invasiveness.

Leafy spurge can form very large dense stands, spreading by seeds, horizontal roots and root pieces distributed by tillage. Seeds are produced in a superior three-celled ovary and released explosively from a seed capsule when it dehisces (Messersmith et al. 1985). Projectile seeds can be launched up to 4.6m from the parent plant (Bowes and Thomas 1978). Up to 250 seeds may be produced by each stem in a growing season (Selleck et al. 1962). Seeds are also transported passively over great distances by wind, running water, animals, humans and equipment (Watson 1985). Although man is the agent in the introduction of seed and root material to the majority of new areas, animals, including both deer and sheep, have been implicated as well. In addition to the likelihood of seeds being stuck to mud on the feet and coats of animals, viable seed has been recovered from sheep manure and new infestations have occurred near spots where deer entrails were buried and accidentally spread by tillage (Selleck et al. 1962). I have also observed Richardson's ground squirrels (*Spermophilus richardsonii*) feeding on and potentially spreading leafy spurge seed. Seeds can remain viable in the soil for at least eight years (Wicks and Dersheid 1964) and seedlings can emerge from depths of 15 cm (Selleck 1962).

Temperature is likely the most important factor influencing germination. Alternating 20 °C and 30 °C resulted in a very high (84%) proportion of germinating seeds while constant temperatures of 30 °C and 20 °C resulted in 74% and 4% germination, respectively (Hanson and Rudd 1933). No seed germination occurred at temperatures between 1 °C and 3 °C (Bakke 1936). A cold period of 2 °C to 5 °C for four weeks preceding a constant 20 °C greatly improved germination (Messersmith et al. 1985). Germination also requires adequate moisture but this factor is secondary to temperature. Alternating wet and dry periods at 48 hr intervals at 20 °C and 30 °C did not significantly influence germination (Selleck 1962).

Assisted by its competitiveness and allelopathy, a single plant can spread by creeping horizontal roots to occupy a 5 m diameter area in one year. Reproductive parts and thus seeds are generally not produced in the first year under field conditions (Selleck et al. 1962). A patch can spread by roots and seed to 0.4 ha in 65 years (Stroh et al. 1990). The median radius increase of spurge patches on uncultivated land was found to be 0.612 m per year and varied with competition and moisture (Selleck et al. 1962).

Leafy spurge. Latex.

Milky latex, although distributed throughout the plant, (Bakke 1936) is contained within specialized elongate secretory cells known as laticifers. This system develops in the embryo and is present in the stem at germination (Messersmith et al. 1985). The longest of these cells extend from root tip to shoot

tip (Lynn and Clevette-Radford 1987, Mahlberg et al. 1987). Latex is released when the plant is mechanically damaged or wounds are opened by herbivores. A variety of functions have been attributed to latex including metabolic and transport roles, wound closure (Lynn and Clevette-Radford 1987, Lym 1998) and, as several acrid compounds are constituents of this substance, a form of protection from phytophagous insects and animals (Lynn and Clevette-Radford 1987).

Toxic constituents include euphorbin, comprised of several phorbol and diterpene esters that cause irritant, inflammatory, purgative, and emetic reactions when consumed. All of these symptoms may occur within the same consumer (Stoner et al. 1987). External contact can cause erythema, desquamation, bulla formation, hair loss and sores on the skin of cattle and humans (Stoner et al. 1987, Lym 1998). Contact with mucous membranes or the eyes is said to be excruciating and has resulted in blindness in livestock and humans (Stoner et al. 1987). Weakness and anecdotal accounts of death have also been attributed to poisoning by ingestion of green plants by cattle (Muenscher 1940). Toxic constituents may also have an indirect effect on humans as they are passed through to the milk of cows (Hausmann and Scurti 1985).

Leafy spurge. Rangeland.

As leafy spurge out-competes forage species and is at very least unpalatable to cattle, weed-infested areas are often avoided by these animals. Moderate to heavy infestations can dramatically depress the carrying capacity of rangeland. Ten percent cover will significantly reduce forage usage by cattle

(Hein and Miller 1992) and heavy infestations can deter it almost entirely. Heavy infestations have resulted in the usage of only 2% of the remaining available palatable plants by cattle (Lym and Kirby 1987).

Leafy spurge. Introduction to North America.

The first documented infestation of leafy spurge in North America was in Newberry, Massachusetts in 1827 (Britton 1921). Contaminated soil ballast, dumped after a ship docked, is considered a probable source of seed for leafy spurge populations on the east coast of North America (Dunn 1985). As a great deal of trade existed between Europe and the east coast of North America at this time, numerous opportunities existed for the introduction of invasive plant species from Europe, including *Euphorbia* spp.

Infestations of leafy spurge in western reaches of North America, however, are more difficult to explain. It is not a simple pattern of western expansion from east coast infestations. There does, however, seem to be a correlation in North and South Dakota, Kansas, Nebraska and Minnesota, between settlement by Mennonites from the mouth of the Dnieper River in the Ukraine and leafy spurge infestations. As these settlers brought wheat and oat varieties from their homeland, the possibility exists that this seed was contaminated with leafy spurge seed (Hanson and Rudd 1933, Dunn 1985). Introductions to Manitoba of contaminated smooth brome grass (*Bromus inermis*) from Germany around 1887 may be responsible for infestations in that province (Dunn 1985). Hutterite elders attribute the introduction of leafy spurge to an area

near the town of Lundbreck in southern Alberta to a shipment of hay from Manitoba during the early part of the 20th century. Recent genetic analysis of leafy spurge populations in North America also suggests, based on similarities of leafy spurge collections with various European *Euphorbia* spp., the possibility of multiple introductions (Rowe et al. 1997).

Leafy spurge. Distribution in Alberta.

Leafy spurge was not recognized as a problem in Western Canada until the early 20th century (Selleck et al. 1962). The distribution of leafy spurge in Alberta as described by Sterling (1956, cited from Selleck et al. 1962) extended from Athabasca, south to the U.S. border and included infestations in the Peace River area. Upwards of 6,000 acres were infested in Alberta in 1995 (McClay et al. 1995). Leafy spurge had spread to over 8,800 acres by 1998 (Figure 1-1). Although the range of the geographic distribution of this weed in Alberta has changed little since the 1950's, the intensity of the problem has increased.

Biological Control of Leafy Spurge.

Leafy spurge control by chemical and cultural means is, though sometimes effective, too expensive, often impractical, and environmentally undesirable in many circumstances. Chemical control of the weed is possible with products like picloram (4-amino-3, 5,6-trichloropicolinic acid). This product is highly phytotoxic in small quantities to most broadleaf, shrub and tree species, is soluble in water, may persist in the soil for 5 years (Ali et al. 2000) and affect sensitive

plants for up to 10 years (Dan Cole *pers. comm.*). Because of these concerns, its use, as well as that of other products, is restricted under the Environmental Protection and Enhancement Act in Alberta to infestations that are more than 30m from bodies of water (Jock McIntosh *pers. comm.*). Prohibitive cost-benefit ratios also deter the use of this and other compounds on many infestations in low quality grazing and natural areas. Mechanical control too is limited by cost and because many infestations occur in rough, inaccessible areas, by topography (Alley and Messersmith 1985, Dersheid et al. 1985, Messersmith and Lym 1995). In addition, shallow cutting by cultivation may actually stimulate shoot development and result in an increase in stem number (Hanson and Rudd 1933).

Because of these issues, and observations that leafy spurge populations are effectively controlled below economic threshold levels in Europe by a large number of herbivores and pathogens, a program to control this weed with European natural enemies was conceived in the early nineteen seventies, with the first release of agents in Alberta during 1978 (Harris 1984, McClay et al. 1995). Other factors which make leafy spurge a suitable candidate for classical biological control include, the weed's non-native status, its taxonomic dissimilarity with native and commercially important plant species, its presence in undisturbed sites so as to facilitate the establishment of control agents and a perennial life history (McClay et al. 1995).

Classical biological control involves the deliberate introduction of natural enemies from the indigenous source of a host species, in this instance, phytophagous insects that feed on leafy spurge in Europe. Once introduced these

agents are expected to colonize the release site, propagate to great numbers and effect control naturally (McClay 1989, Pedigo 1989).

The first biological control agents released were the spurge hawk moth, *Hyles euphorbiae* (Sphingidae), the root moths, *Chaemaesphesia empiformis* and *C. thenthrediniformis* (Sesiidae) and the longhorn beetle, *Oberea erythrocephala* (Cerambycidae). Both *H. euphorbiae* and *O. erythrocephala* established but had little effect on leafy spurge infestations (LeSage and Paquin 1997).

Exploration throughout central Europe in the late nineteen seventies revealed approximately 40 species of flea beetles (Coleoptera: Chrysomelidae: Alticinae) in the genus *Aphthona* Chevrolat found in close association with European populations of *Euphorbia* spp. (Nowierski et al. 1996). After a careful screening process to assess the potential host range and subsequent risk to native North American spurge species and commercially important plant species, these insects received approval under the Canadian Plant Protection Act after being individually reviewed by an advisory panel of Agriculture and Agri-Food Canada. Five species were approved for release: *A. nigriscutis*, *A. cyparissiae*, *A. lacertosa*, *A. czwalinae* and *A. flava* (McClay 1995).

Aphthona spp.

Adult *Aphthona* are characterized by: front carinate, frontal tubercles well developed and clearly margined, antennae longer than half the body length, prothorax broader than long, pronotum lacking both longitudinal and transverse grooves, elytra wider at base than pronotum, elytral punctuation confused or,

sometimes, in irregular rows, procoxal cavities open, tibial spurs simple and inserted at outer corner of tibia, first hind tarsi usually distinctly shorter than half length of hind tibia (Chevrolat 1837, cited from LeSage and Paquin 1997).

Identities of adults of the *Aphthona* spp. released in Canada can be determined by colour, though some overlap does occur among the yellow species, *A. nigriscutis*, *A. cyparissiae* and *A. flava* and between the black species *A. lacertosa* and *A. czwalinae*. *A. nigriscutis* can be distinguished from the other yellow species by their darker, almost brown colouration and black scutellum (Rees et al. 1996). For more accurate identification, the morphologies of aedeagi of males and the spermathecae and styli of the females must be examined (LeSage and Paquin 1997).

Sexual dimorphism is evident. Males have an enlarged first segment of the front tarsus, a distinctly lobate last visible abdominal sternite, are on average smaller and more slender and have antennae that are proportionally longer, with thicker segments, than females. In yellow species like *A. nigriscutis*, males also have a median dark line on the last visible abdominal sternite (LeSage and Paquin 1997).

Aphthona spp. Life History.

All of the *Aphthona* spp introduced to Canada have univoltine life cycles and adults are small insects, between 2 and 3 mm in length. They feed upon the foliage and flowers of leafy spurge and larvae feed upon roots and root hairs. Adults emerge from the soil in late spring or early summer and can be present on

a site for several weeks to months (*pers. observ.*). Eggs are laid by mated females near or on the bases of leafy spurge shoots (Hansen et al. 1997). Female *A. nigriscutis* can produce more than 500 eggs, laid in clumps over a period that can exceed 100 days (Jackson 1997). Immediately after hatching, larvae burrow into the soil and feed upon fine root hairs. There are three larval instars and larger root structures are utilized as larval size increases. Large larvae tunnel through the root system (Maw 1981). Pupation occurs in the spring, in a cell constructed in the soil near the host plant roots (Hansen et al. 1997).

Although adults may completely defoliate host plants, this damage has been suggested to have little impact on plant survival. Larval feeding upon root hairs and roots influence plant uptake of nutrients and water and allow, through feeding wounds, points of entry for soil-borne pathogens (Hansen et al. 1997). In addition, sites with previous larval feeding are preferred, resulting in aggregated larval distributions and high levels of localized damage to roots (Gassman et al. 1996). Collectively, this combination of larval impacts can lead to plant death.

Introduction of A. nigriscutis to Canada

The first introductions of *Aphthona nigriscutis* obtained from Hungary to North America were made near Cardston, Alberta in 1983. This population of beetles flourished and was the source of 25,000 beetles between 1985 and 1992 (McClay et al. 1995). These beetles were redistributed to approximately 130 sites in Alberta, including the Beverly Bridge sites in Edmonton. The Edmonton sites

have been the source of more than 100,000 beetles since 1994 (Stromme et al. 2000).

Beetles established upon 69.5% of the releases made between 1983 and 1995 (McClay et al. 1995). Some of the factors thought responsible for inconsistent establishment include soil texture, soil pH and organic matter content (McClay et al. 1995) as well as target weed density and canopy height (Rees et al. 1996, Spencer 1996). Acidic soils and high moisture and organic matter content were associated with the failure of these beetles to establish on some sites. Plant height below 18 in and low spurge density were associated with successful beetle establishment and weed suppression. Mechanisms for the effects of these factors have yet to be examined. Variability of the intended host plant may also influence establishment (for example: Shultz-Schaeffer and Gerhardt 1989, McClay et al. 1995).

Host Variability.

There is some controversy concerning the taxonomic status of North American leafy spurge(s). Moore (1958) and Pritchard (1961) concluded that all North American leafy spurge was comprised of one polymorphic species, *E. esula* L.. Radcliff-Smith (1985), using morphological features conservatively described at least twenty species taxa and hybrid combinations. A recent morphological treatment again indicated that all North American leafy spurge should be considered one variable species but also indicated that three other spurge species should be recognized (Crompton et al. 1990). Crompton et al. (1990) suggest that

the description of greater numbers of species may be due to excessive splitting, symptomatic of what they consider to be a historical trend that has characterized recent European taxonomy. One criterion used to morphologically distinguish taxa is the leaf width ratio. Mahlberg et al. (1987) found that this character can vary significantly between leaves from the same plant making distinctions between taxa based upon this character potentially inaccurate.

Some cytotaxonomic analysis supports the hypothesis that North American leafy spurge is a variable species complex of *E. esula* and *E. x pseudovirgata* with a possible contribution from *E. cyparissias* (Schulz-Schaeffer and Gerhardt 1989). Moore (1958) also described a hybrid between *E. esula* and *E. cyparissias*. Genetic analysis also indicated substantial differences between and within weed populations. These differences were great enough to suggest the possibility of multiple introductions to North America but could not exclude the possibility of a high degree of variability of source populations in Europe (Rowe et al. 1997). Another cytogenetic study by Stahevitch et al. (1988) that examined 125 leafy spurge accessions for chromosome number, meiotic irregularities and failure to hybridize indicated that these samples were all members of one polymorphic species: *E. esula* L.

Differences in the latex triterpenoid profiles of North American accessions have also been detected among sites. The latex triterpenoid profiles of a number of European *Euphorbia* spp. have been shown to be stable and are considered diagnostic of *E. amygdaloides*, *E. agraria*, *E. cyparissias*, *E. lucida* and *E. seguieriana* regardless of the collection site. Thirty-seven European collections

of *E. esula*, however, were divisible into 15 groups. Their profiles were compared to those of North American leafy spurge from 39 sites. The North American leafy spurge tested was less variable than the European *E. esula* and the profiles of all but one distinctly North American collection were placed into 3 of the 15 groups (Holden and Mahlberg 1992). These results suggested that *E. esula* is highly variable in Europe and that this variability extends in some part to North American populations. It also suggests that North American leafy spurge may be comprised predominantly of a single, highly variable species and that some populations in North America may have diverged or hybridized to form distinctly North American groups.

North American leafy spurge may be a highly variable species complex or one highly variable species. Regardless of the lack of consensus regarding the taxonomic status of North American leafy spurge populations, there is some agreement on its variability.

Part of this variability extends to the leaf wax triterpenoid profiles of North American leafy spurge. The epicuticular waxes of four North American and one Austrian leafy spurge collection were extracted and their profiles compared. Variations in the amounts of the triterpenoids α -amyrin, β -amyrin, δ -amyrenone, 24-methylenecycloartenol and lupeyl acetate were detected both among the North American samples and between North American and Austrian samples. All of the North American samples contained high levels of α -amyrin, low levels of β -amyrin and moderate amounts of δ -amyrenone. The Austrian spurge had moderate levels of α -amyrin, high levels of β -amyrin and no δ -

amyrenone. All but one North American sample contained high levels of 24-methylenecycloartenol. This collection and the European collection both shared considerably lower lupeyl acetate levels than the other plants tested (Manners and Davis 1984). In addition to further illustrating the variability of leafy spurge in North America and its potentially hybrid nature this study also illustrated that differences occur in surface compounds, like α -amyrin and β -amyrin, that have been shown to influence the host preferences of other insect species.

The triterpenoids α -amyrin and β -amyrin were found to be present in higher concentrations in azalea varieties resistant to the azalea lace bug *Stephanitis pyrioides* (Heteroptera: Tingidae) (Balsdon et al. 1995) and crucifers resistant to diamondback moth larvae, *Plutella xylostella* (Lepidoptera: Plutellidae) (Eigenbrode et al. 1991) than in susceptible varieties. It is possible that the variable concentrations of these compounds may also affect host selection by *A. nigriscutis* and impart anti-xenosis resistance to some leafy spurge collections.

There are, in so far as the responses of insects intended to be biological control agents are concerned, potentially relevant differences between some North American leafy spurge collections and European spurge species. Collections and larval survival tests indicated that *E. esula* (*sensu stricto*, from European collections), *E. cyparissias* and *E. seguieriana* may be more appropriate host plants for these beetles than the North American leafy spurge tested (Gassman et al. 1996). Collections of *A. nigriscutis* in Europe indicated a close association of *A. nigriscutis* adults with *Euphorbia cyparissias* and *E. seguieriana* in the field

(Maw 1981, Gassman et al. 1996). Larval survival tests indicated differences in the suitability of several spurge species, including North American leafy spurge, as hosts for these beetles. First instar beetle larvae were transferred to North American leafy spurge, *E. cyparissias* and *E. seguieriana*. Roughly three times more larvae survived until the third instar upon *E. esula* and *E. seguieriana* than on the North American leafy spurge tested (Gassman et al. 1996). Although *A. nigriscutis* larvae did not completely reject the North American leafy spurge tested, the differences in larval survival may indicate antibiosis resistance in this collection. Other potential biological control agents, though found in close association with *E. esula* from Austria and Switzerland, rejected the North American collections tested completely (Harris et al. 1985). Although differences in the suitability of various North American collections of leafy spurge to established agents like *A. nigriscutis* have also been hypothesized, they have yet to be tested.

Potential Influence of Host Variability on A. nigriscutis.

Host variability may influence host selection in a number of ways. Variation in surface semiochemicals associated with host selection may influence the feeding responses of herbivorous insects. The triterpenoids α -amyrin and β -amyrin influence host choices of *Stephanitis pyrioides* (Heteroptera: Tingidae) (Balsdon et al. 1995) and diamondback moth larvae, *Plutella xylostella* (Lepidoptera: Plutellidae) (Eigenbrode et al. 1991).

There may be variability in plant compounds that serve as pheromone precursors. Production of components of *Ips paraconfusus* (Coleoptera: Scolytidae) aggregation pheromone requires exposure to the host tree monoterpene, myrcene (Hughes 1974). Variability may also extend to plant produced pheromone components. Females of the European elm bark beetle, *Scolytus multistriatus* (Scolytidae) produce an aggregation pheromone. This pheromone consists of 4-methyl-3-hepanol and (-)- α -multistriatin, both produced by *S. multistriatus* and (-)- α -cubebene, produced by the host tree (Gore et al. 1977, cited from Lanier 1983, in Ahmad 1983). Lanier (1983, in Ahmad 1983) suggests that potential host trees that do not produce (-)- α -cubebene when attacked do not contribute to the mixture and are not extensively colonized.

Variability may also extend to plant volatile compounds potentially used by *A. nigriscutis* as host-association cues and further influence the attractiveness of various leafy spurge biotypes. A biotype is defined as a classification of organism that is morphologically similar to but physiologically different from other members of the species (Arneson 2001). *Diabrotica* spp. (Chrysomelidae) are attracted to host plant volatiles, tetracyclic triterpene cucurbitacins. These compounds act as arrestants and feeding stimulants for these cucurbit specialists. Attack and feeding are directly related to the amounts of cucurbitacins B and E (Metcalf et al. 1979).

As a consequence of this variability, aggregation and dispersal behaviours of *A. nigriscutis* may be affected. Aggregation behaviour, in particular, as a means of localizing damage and promoting secondary infections associated with

their feeding, has been implicated as important to the success of biological control agents. The success rate of biological control agents that aggregate in the field is greater than that of agents that do not (Begon et al. 1990, p.352).

A. nigriscutis Aggregation and Dispersal Behaviour

A. nigriscutis have been shown to assume aggregated distributions in the field. At some sites, adult beetles completely defoliate the plants on the original point of release and radiate outward as a “solitary population wave” in a roughly circular pattern. This can only occur in sufficiently large stands or where the architecture of the stand permits this movement. Beetle density is highest and many hundreds of beetles congregate on the wave front (Kovalev 1990, cited from McClay et al. 1995). On other sites, once beetles are established, they disperse to form new colonies near the original point of release (Bob Carlson, *pers. comm.*). I have also observed an aggregated distribution of beetles when collecting these insects. In Edmonton very large numbers of beetles were found only at some points collected along a strip of leafy spurge at the top of a hill despite the relatively consistent density of plants along this strip. The distribution of *A. nigriscutis* immediately after release has yet to be examined.

Fadamiro and Wyatt (1996) demonstrated that *Prostephanus truncatus* Horn (Coleoptera: Bostrichidae) dispersal behaviour was affected by resource suitability. Beetles were more likely to disperse from poor quality food sources. The effects of crowding and resource depletion on dispersal have also been demonstrated for the goldenrod beetle, *Trirhabda virgata* (Coleoptera:

Chrysomelidae). Female *T. virgata* were more likely to disperse both when male beetles were absent and when host plants were heavily defoliated (Herzig 1995). Herzig and Root (1996) suggest that this dispersal of females from heavily defoliated plants is likely a strategy to disperse their offspring to areas that have not been subject to over-exploitation. The observation that female dispersal was greater without males may also suggest the influence of a male-produced aggregation pheromone upon these chrysomelids.

A. nigriscutis may also respond to crowding by dispersing. On the Cardston site, beetles were found primarily within 5 to 10 m of the original release point for the first few years. As the population grew, the speed of dispersal increased and adult beetles were found 100 m from the site four to five years after release. The mean dispersal distances from all established *A. nigriscutis* sites in Alberta, 2, 3 and 4 years after release, were 14 m, 66 m and 124 m respectively (McClay et al. 1995). This increasing dispersal rate shown for *A. nigriscutis* on Alberta release sites may indicate that these beetles also respond to crowding by dispersing. The mechanisms of aggregation and dispersal for *A. nigriscutis* have yet to be examined but may include conspecific chemical cues, plant volatiles and synergistic effects of these compounds.

The behaviours and distributions of number of beetles are affected by conspecific chemical cues. These are volatile compounds that elicit a response in members of the same species, pheromones. For example, *Maladera matrida* Argaman (Scarabaeidae) (Yarden and Shani 1994), *Phyllotreta cruciferae* Goeze (Chrysomelidae: Alticinae) (Peng and Weiss 1992) and *Longitarsus jacobaeae*

(Chrysomelidae: Alticinae) (Zhang and McEvoy 1996). All of these beetles are affected by aggregation pheromones. The host plant may influence the production of these cues. Contact with the host plant is necessary for the production of the cues for *Phyllotreta cruciferae* Goeze (Peng and Weiss 1992). Some beetles are also influenced by a synergy or additive effect of plant volatiles and conspecific cues, for example, *Carpophilus hemipterus* (Nitidulidae) (Dowd and Bartelt 1991). The attraction of these beetles to their aggregation pheromone is augmented by plant volatiles.

According to Andrewartha and Birch (1954), subpopulations that are spatially separated can fluctuate independently and, should a local extinction event occur, can be repopulated by immigrants from nearby subpopulations. They described populations as shifting mosaics. The dynamics of metapopulations, small isolated groups or subpopulations of *A. nigriscutis* may be influenced by host variability and its effects upon plant volatiles associated with host recognition and the production of conspecific cues.

The distribution of variable leafy spurge plants may also influence *A. nigriscutis* distribution. Should more than one, including an attractive biotype populate a site, more beetles may be attracted to it than the others at this site. This selective attraction to and aggregation upon select host plants of the same species on the same site has been demonstrated for the *Eucalyptus stelulata* and *E. pauciflora*-feeding *Chrysophtharta hectica* (Coleoptera: Chrysomelidae) (Strauss and Morrow 1988). Selective attraction of beetles to plants of a certain biotype

on a site may also have implications for the metapopulation dynamics of *A. nigriscutis*.

Project Synopsis.

Although *A. nigriscutis* has established in North America, the success of this agent has been inconsistent and may be influenced by the variability of its intended host plant. The variability of leafy spurge in North America has been demonstrated, though its influence on biological control agents has not. I have chosen to examine this variability as determined by the responses of *A. nigriscutis* to leafy spurge collected from a number of sites throughout southern and central Alberta. Plants collected from several points along transects through several sites were tested in a manner similar to that used by Palaniswamy et al. (1992) to assess antixenosis resistance of canola, *Brassica napus* L. and *B. campestris* (= *B. rapa*), varieties to *Phyllotreta cruciferae*. The feeding preferences of *A. nigriscutis* to leafy spurge plants collected from a number of locations were compared. Olfactometer tests were also used to indirectly determine the effects of hypothetically variable volatile leafy spurge compounds, beetle conspecific cues and the effects of plants from several sites on the production or reception of these cues. In addition, field tests were used to assess the distribution of beetles on leafy spurge stands and the effects of hypothesized host variability on these distributions shortly after release. Differences in the responses of beetles to various collections of North American leafy spurge may provide evidence for host plant variability as a factor that influences *A. nigriscutis* host choices, spatial

distribution and subsequently, the success of this agent as a biological control agent.



Figure 1-1. Municipal Districts, Improvement Districts, Special Areas and Counties in Alberta with leafy spurge infestations. This information was compiled with the assistance of Dennis Laughton of the Waldern Grazing Reserve and District Agricultural Fieldmen in 1998.

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Chapter 2

Feeding Preferences of *Aphthona nigriscutis*.

Introduction

The black dot flea beetle, *Aphthona nigriscutis* Foudras has been an effective classical biological control agent of leafy spurge, *Euphorbia* spp. near *esula*, on some sites. One established beetle population in Edmonton, Alberta reduced target weed stand density by 99%, allowing for a 30-fold increase in grass biomass in four years (Stromme et al. 2000). Although many release sites now have established populations of *A. nigriscutis* and some of these have suppression of leafy spurge, significant proportions do not (McClay et al. 1995, Hansen et al. 1997).

Some of this variability in *A. nigriscutis* establishment may be attributed to site factors. This beetle is usually found on dry, open sites with coarse, sandy soils in Europe (Gassman et al. 1996). *A. nigriscutis* larvae are unable to tolerate relatively high moisture levels or loamy soils and therefore establish less frequently on North American release sites with these conditions (Rees et al. 1996). Establishment may also be subject to plant community parameters such as target weed density and canopy height. Plant height below 18 in and relatively low spurge density are associated with successful beetle establishment and weed suppression (Rees et al. 1996, Spencer 1996). Soil texture, organic matter and pH may also influence beetle establishment. Acidic soils and high soil moisture and organic matter content have been associated with the failure of these agents to establish (McClay et al. 1995).

Differences between North American leafy spurges and the European spurge species that are the sources of biological control agents may also influence

establishment. *A. nigriscutis* is found in close association with *E. cyparissias* and *E. seguieriana* in Europe (Maw 1981, Gassman et al. 1996). The spurge species complex considered predominant in Canada by Harris et al. (1985) is *E. x pseudovirgata*.

Larval survival tests indicated differences in the suitability of these spurges as hosts for beetles. First instar *A. nigriscutis* larvae were transferred to North American leafy spurge, *E. cyparissias* (sensu stricto) and *E. seguieriana*. Roughly three times more larvae survived until the third instar upon *E. esula* and *E. seguieriana* than on the North American leafy spurge tested (Gassman et al. 1996). Although *A. nigriscutis* larvae did not completely reject the North American leafy spurge tested, the differences in larval survival may indicate antibiosis resistance.

There are also differences in the responses of other spurge specialist insects to *Euphorbia* species. The root boring moth, *Chamaesphecia tenthrediniformis*, attacks *E. esula* (sensu stricto) but not *E. virgata* despite their similar morphologies and the ecological niche shared by both plant species. These spurge species occur in close proximity in European stands but oviposition occurs only upon *E. esula*. Newly hatched *C. tenthrediniformis* larvae become paralyzed by contact with small amounts of the latex of *E. x pseudovirgata*. In addition, attempts to establish the *E. esula* specialist aphid, *Acyrtosiphon neerlandicum* in quarantine on *E. x pseudovirgata* failed (Harris et al. 1985). Harris et al. (1985) attribute differences in the responses of specialist insects to

various *Euphorbia* spp. to chemical differences in the latex and toxins of these spurge species.

Chemical differences among the latexes of North American and European spurges have been shown. Holden and Mahlberg (1992) found that the latex triterpenoid profiles of samples of leafy spurge from 39 North American accessions could be grouped into four categories. Three of these shared similarities with categories of European *E. esula* but one was distinctly North American. Properties of these triterpenoid compounds, relevant to herbivore host selection and feeding, have yet to be examined.

Variability also extends to the leaf surface chemistry of leafy spurges. The epicuticular waxes of four North American and one Austrian leafy spurge sample were extracted and their profiles compared. Variations in the amounts of the triterpenoids α -amyrin, β -amyrin, δ -amyrenone, 24-methylenecycloartenol and lupeyl acetate were detected both among the North American samples and between North American and Austrian samples. All of the North American samples contained high levels of α -amyrin, low levels of β -amyrin and moderate amounts of δ -amyrenone. The Austrian spurge had moderate levels of α -amyrin, high levels of β -amyrin and no δ -amyrenone. All but one North American sample contained high levels of 24-methylenecycloartenol. This sample and the European both shared considerably lower lupeyl acetate levels than the other plants tested (Manners and Davis 1984). In addition to further illustrating the variability of leafy spurge in North America and its potentially hybrid nature this study also illustrated that differences occur in surface compounds, like α -amyrin

and β -amyrin, that have been shown to influence the host preferences of insects on other plants.

The triterpenoids α -amyrin and β -amyrin were found to be present in higher concentrations in azalea varieties resistant to the azalea lace bug *Stephanitis pyrioides* (Heteroptera: Tingidae) (Balsdon et al. 1995) and crucifers resistant to diamondback moth larvae, *Plutella xylostella* (Lepidoptera: Plutellidae) (Eigenbrode et al. 1991) than in susceptible varieties. The concentrations of these compounds may also affect host selection by *A. nigriscutis* and impart anti-xenosis resistance upon some leafy spurge biotypes.

Although there is little consensus regarding the taxonomic status of North American leafy spurge there is some agreement regarding its variability. North American leafy spurge may be one highly polymorphic species or a hybrid species complex. Its highly variable nature may be a result of hybridization subsequent to multiple introductions of *Euphorbia* spp. in North America and / or may be due to that of variable source populations in Europe (Moore 1958, Pritchard 1961, Radcliff-Smith 1985, Manners and Davis 1984, Mahlberg et al. 1987, Schultz-Scheaffer and Gerhardt 1989, Crompton et al. 1990, Holden and Mahlberg 1991, Rowe et al. 1997). If differences in the latex and toxins of these spurge species influence the host choices of or suitability to specialist herbivores, as suggested by Harris et al. (1985), the variability of leafy spurge among North American sites may influence the establishment of some biocontrol agents.

Rowe et al. (1997) found significant genetic differences among plants collected from several North American leafy spurge accessions. They examined

maternally inherited chloroplast DNA to assess the relatedness of these leafy spurges. The differences they found were profound enough to suggest the possibility of multiple introductions of different spurge species.

Rowe et al. (1997) also demonstrated that significant differences occur among plants from the same populations. Most of the collection sites that they examined were dominated by one biotype of leafy spurge but also supported several less frequently occurring biotypes. A biotype is defined as a classification of organism that is morphologically similar to but physiologically different from other members of the species (Arneson 2001).

Within-site variability may also influence *A. nigriscutis*. Specialist herbivores discriminate amongst potential hosts on mixed-biotype stands. This selective attraction to and aggregation upon select host plants of the same species on the same site has been demonstrated for the *Eucalyptus stelulata* and *E. pauciflora*-feeding *Chrysophtharta hectica* (Coleoptera: Chrysomelidae) (Strauss and Morrow 1988). The occurrence of multiple *Euphorbia* species or biotypes on one site may also influence the distribution of herbivores like *A. nigriscutis*.

A. nigriscutis assume aggregated distributions in the field. At some sites, adult beetles completely defoliate plants on the original point of release and radiate outward in a roughly circular pattern consistent with the “solitary population wave” described by Kovalev (1990, cited from McClay et al. 1995) for *Zygogramma suturalis* (Chrysomelidae). Where *A. nigriscutis* populations are large, density is highest and many hundreds of beetles congregate on the wave front (*pers. observ.*). Host plant factors such as surface, latex or toxin chemistry

that affect host choices could influence beetle distribution. Host discrimination in a heterogeneous patch may promote aggregation behaviour. As aggregation behaviour has been implicated as a factor that contributes to the success of some biological control agents (Beddington et al. 1978, cited from Begon et al. 1990), factors that affect this behaviour may influence the impact of *A. nigriscutis*.

For this study, plant samples were collected from ten beetle release sites in the central and southern locations in Alberta. Collection site selection was based upon monitoring data from these sites. The numbers of *A. nigriscutis*, at least three years after release, per sweep net sample, were used as a measure of establishment. Feeding and oviposition preferences of *A. nigriscutis* and between and within-site variability of feeding preferences were assessed. The correlation of overall palatability of leafy spurge from each site, variability of the feeding preferences at each site, as well as that of the site parameters, leafy spurge height and density and soil texture as determined by silt, sand and clay content, as well as soil pH and organic matter content with *A. nigriscutis* establishment were also assessed.

Materials and Methods

Plants.

Plant Collection and Propagation, 1998. In preparation for testing the feeding preferences of *A. nigriscutis* among various leafy spurge samples, plants were collected from nine large weed infestations in eight municipalities in southern and central Alberta during May 1998. Samples were taken from sites in or near Edmonton, Ponoka, Hardisty, Olds, Fort Macleod, Lethbridge, Millet, and from two sites near Cluney, Alberta (Figure 2-1). Beetles had been released on each of these sites at least seven years before this study and regularly monitored for at least three years following their release. Beetle establishment, based on the maximum numbers of beetles that were recovered in subsequent years, varied from site to site (unpublished data, courtesy Alec McClay) (Table 2-1). These beetle recoveries were used to categorize each site into one of three establishment classes - *good* with 100 or more beetles in 20 sweeps, *moderate* with 6 to 99, and *poor* with 0 to 5 beetles.

Plants were collected from three sites from each establishment category. In addition, leafy spurge plants were also obtained from Dr. Alec McClay of the Alberta Research Council, Vegreville (hereafter referred to as ARC). These plants were originally collected from a well-established *A. nigriscutis* release site near Cardston, AB in 1991 and had been used since to rear and maintain leafy spurge biological control agents. These plants are hereafter referred to as Cardston (ARC) leafy spurge plants.

Individual plants were excavated. Plant top-growth was removed and intact, approximately 2700 cm³ clumps of soil and root material were transported to a greenhouse where roots and soil were separated. To reduce effects of individual variation, all plants tested from each site were grown from cuttings of a single large root from each sample. A single plant provided all of the root material for propagation of Cardston (ARC) plants. Plants propagated from each sample were clones. The cut, approximately 2 cm long by 5 mm diameter leafy spurge roots were planted in a moistened commercial growth medium (Sunshine Professional™) in a 15 cm diameter pot. Each large root was divided into at least ten portions to ensure adequate material for testing.

Once plants had grown to a height of approximately 30 cm, their growing tips were cut to promote the growth of lateral buds and increase the amount of harvestable plant material. Once lateral outgrowths had grown to at least 5 cm, they were removed, their severed ends were dipped in indole butyric acid rooting hormone and they were transferred to growth medium in 7 dram plastic vials (Figure 2-2). Each vial had a small hole in its bottom to allow for sub-irrigation. Plants were maintained in very moist growth medium for at least one week to ensure that they were turgid and to allow rooting to begin. Plants collected in 1998 were maintained in the greenhouse over the winter and tested again in 1999.

Plant Collection and Propagation, 1999. In order to assess the within-site variability of leafy spurge palatability to *A. nigriscutis*, an additional 10 plants were collected from each site, including the Cardston site that was the original

source of Cardston (ARC) plants, in early May 1999. At most sites plants were sampled at approximately 5 m intervals along linear transects through each spurge population. Each transect was placed in an effort to incorporate the estimated 1998 sampling sites at a roughly mid-transect point.

Plants were sampled along two transects at the Cardston and Edmonton sites. The Cardston site was sampled along two transects that were approximately 100 m apart. The precise sampling point where Cardston (ARC) leafy spurge was initially collected was unknown so it could not be deliberately incorporated into either transect. Leafy spurge density has been greatly reduced at the release points of the Edmonton site so plants were sampled along two transects approximately 20 m west of this site. Plants were sampled along two 25 m transects along the top and bottom of a south-facing hillside with pronounced suppression of the leafy spurge mid-slope.

Plants were collected and propagated in the same manner as those in 1998. Lateral stem cuttings, however, were rooted in sand rather than the commercial growth medium. This rooting medium allowed sifting to ease the detection and recovery of *A. nigriscutis* eggs from vials.

Beetles.

1998. Adult *A. nigriscutis* were collected with sweep nets near Bozeman, Montana by Richard Hansen (USDA/APHIS) and shipped to Edmonton. The leafy spurge samples tested in 1998 were tested again in 1999 with *A. nigriscutis* collected in Edmonton.

1999. Adult *A. nigriscutis* were collected from the Beverly Bridge site in Edmonton. Collections were made at approximately bi-weekly intervals from late June to late September of 1999. All beetles were maintained on Cardston (ARC) plants in 60 by 60 by 120 cm, wooden frame, nylon mesh cages for at least 24 hr before each test. Beetles participated in tests only once.

Laboratory Bioassay. Methods for this bioassay are similar to those used by Palaniswamy et al. (1992) to assess antixenosis resistance of canola, *Brassica napus* L. and *B. campestris* (= *B. rapa*), varieties to *Phyllotreta cruciferae*. Small cuttings rooted in seven-dram vials were transferred to evenly spaced holes in a 54 by 54 by 3 cm foam plastic plate (Figure 2-3). This plate served as the floor of a feeding trial arena. Arenas had internal dimensions of 54 by 54 by 30 cm high and were topped with nylon mesh. Plexiglas™ walled arenas were used in 1998 and glass-walled arenas with the same dimensions were used in 1999 (Figure 2-4). The glass walls could be easily cleaned with ethanol between runs and were less likely to retain volatile compounds.

Ten clones each from ten samples were tested at a time and placed in a randomized Latin square array (i.e. each sample was represented once, in random order in each row and each column) (Table 2-2). The test was replicated four times in 1998. Most tests were replicated four times in 1999 but as some of the leafy spurge samples taken in 1999 provided inadequate plant material for four replicates, were replicated as many times as available plant material would allow.

Plants from each sample were randomly assigned to tests and were not necessarily tested against other plants from the same site (Table 2-3).

A. nigriscutis ($n=400$ in 1998 and $n=1000$ in 1999) were introduced to the feeding trial arena through a capped port in one wall in 1998 and through a removable top in 1999. They dispersed rapidly through the arena. The beetles were allowed to feed for a prescribed time, 6 days in 1998 and 2 days in 1999. The numbers of *A. nigriscutis* were increased in 1999 to shorten the test running time and allow a larger number of plants to be tested. When the allotted time had elapsed, the beetles were removed from the arena and the amount of feeding damage to each plant was assessed. Feeding damage to each plant was rated on a scale of 0-9: 0 representing 0-10% defoliation, 9 representing 90-100%. Plants that were collected in 1998 were maintained in a greenhouse over the winter and tested again in 1999. The eggs deposited by *A. nigriscutis* on each plant and in the growing medium in each vial were counted in 1998 and for the first test of 1999.

All tests were conducted in a continuously illuminated room at $20 \pm 3^{\circ}\text{C}$. *A. nigriscutis* converge on light sources when confined (*pers. observ.*), so arenas were placed squarely below evenly spaced fluorescent fixtures in the laboratory to minimize directional effects of lighting.

Relating Damage Ratings to Beetle Establishment. The correlation of overall palatability of the spurge sampled from each site, the mean feeding damage ratings of all samples from each site, and beetle numbers were assessed. The

correlation of within-site variability of feeding damage with beetle establishment was also assessed. Within-site variability was characterized by the variance (Snedecor and Cochran 1971) of the transformed feeding damage ratings of all samples from that site.

The correlation of each site's soil pH, organic matter, sand, silt and clay content (unpublished data, courtesy Alec McClay) (Tables 2-6 and 2-7) to beetle numbers was also assessed.

Data Analysis.

Feeding Trials. Feeding damage ratings were transformed by arcsine ($\sqrt{(x + 0.5)}/10$) to stabilize the variances. Comparisons of feeding damage ratings were made by ANOVA within sites, between sites, and between the 1998 and 1999 damage ratings of the 1998-collected plants. Comparisons of the mean numbers of eggs laid on or near the plants collected in 1998 in both 1998 and 1999 were also made using ANOVA. When significant differences were detected (at $\alpha=0.05$), means were separated using Student-Newman-Keul's test.

Correlation of Site Factors to Establishment. The correlation of overall palatability of leafy spurge from each site, variability of the feeding preferences at each site, as well as that of the site parameters, leafy spurge height and density and soil texture as determined by silt, sand and clay content, as well as soil pH and organic matter content with *A. nigriscutis* establishment were assessed using a Spearman Rank Correlation.

Results

Plants. Plant sample clones began sprouting from root cuttings within one week and most grew to a height of 30 cm in four weeks. Very few of these plants produced flowers.

Feeding Trials, 1998. There were significant differences in the level of feeding damage among samples from these sites ($p=0.0001$). Ponoka plants were most heavily damaged and Cardston (ARC) plants were the least (Table 2-4). There was also a significant row ($p=0.0090$) effect in this test. There were no significant differences ($P=0.0823$) in the numbers of eggs laid on or near plants from each site.

Feeding Trials, 1999. Significant differences in *A. nigriscutis* feeding preferences for plants collected in 1998 were again apparent when these plants were examined in 1999 ($p=0.0001$). There were no significant differences between the feeding damage ratings of 1998 and those on the same plants in 1999 (comparison of both tests: $p=0.0804$). Ponoka plants were again the most heavily damaged and Cardston (ARC) plants were the least (Table 2-5). There was a significant column effect ($p=0.0016$) and a row effect approached significance ($p=0.0527$). There were significant differences in the feeding damage per column ($p=0.0190$) in the first replicate of this test. There were also significant row and column effects in the third replicate ($p=0.0332$, $p=0.0226$) (Figure 2-5). There were no edge effects.

There were no significant differences in the numbers of eggs laid by plant sample in test 1, 1999 ($p=0.1036$).

There were significant between-site differences when the feeding damage ratings on samples from all sites were compared ($p=0.0001$). Overall, plants collected from the Hardisty site were the most heavily damaged while those from the Millet site were the least (Table 2-6). There were no significant within-site differences among the samples collected from Cluney 2, Ponoka, Hardisty, Olds, Millet, Lethbridge or Fort Macleod at $\alpha=0.05$. The Edmonton site was the most variable and the Millet site was the least. The variability of the feeding damage ratings to all of the plants from each collection site is summarized in Tables 2-7 and 2-8.

Relating Damage Ratings to Beetle Establishment. Of the factors examined, the only ones that were significantly correlated by Spearman's rank correlation with beetle numbers were soil pH ($r=0.7139$, $p=0.0204$) and within-site variance ($r=0.7312$, $p=0.0165$) (Table 2-9).

Discussion

There were significant row and column effects in some replicates of these tests. There were no edge effects. The areas of heaviest damage were localized, suggesting that beetles formed aggregations within each test arena.

Despite localized feeding, there were still significant differences in the level of feeding damage to leafy spurge samples from each of the collection sites. There were also significant differences among *A. nigriscutis* feeding preferences for plants collected from different points on these sites. Such differences indicate that variability in some character(s) that influences the feeding preferences of *A. nigriscutis* occurs both between and within populations of leafy spurge in Alberta.

Differences in feeding preferences were retained regardless of the numbers or immediate source population of beetles that fed upon the plants or the growth medium used to root plant cuttings. These results demonstrated that differences between the host preferences of the two source populations of *A. nigriscutis* are negligible and, as differences were maintained for over a year, are stable, and are likely due to genetic variability among these leafy spurge samples. Rowe et al. (1997) demonstrated such variability among their study leafy spurge populations. A biotype is defined as a classification of organism that is morphologically similar to but physiologically different from other members of the species (Arneson 2001). Variable feeding preferences of *A. nigriscutis* likely indicate physiological differences among these leafy spurge samples.

Although this has yet to be tested, variable *A. nigriscutis* feeding preferences may indicate the influence of a feeding deterrent. Manners and Davis

(1984) demonstrated differences in the leaf wax contents of the triterpenes α -amyirin and β -amyirin among leafy spurge populations. As the concentrations of these compounds in host plant tissues have been shown to influence the feeding and oviposition responses of other insects (Eigenbrode et al. 1991, Balsdon et al. 1995), they may also influence those of *A. nigriscutis* and impart anti-xenosis resistance upon some leafy spurge biotypes.

Other variable plant compounds may be responsible for the attraction of *A. nigriscutis* to select plants, arresting beetles on these plants and initiating and sustaining feeding behaviours. *Cucurbita* spp.- feeding *Diabrotica* spp. (Chrysomelidae) respond to the amounts of cucurbitacins B and E in their host plants. Attack and feeding of these beetles are directly related to the concentrations of cucurbitacins B and E (Metcalf et al. 1979). Spurge specialist *A. nigriscutis* may also respond to compounds with similar roles in *Euphorbia* spp..

Compounds associated with the nutritional value of the plants also influence host selection behaviours of insects. Mitchell (1974) found that the leaf surface compositions and concentrations of amino acids influenced the gustatory responses and subsequently the host selection of *Leptinotarsa decemlineata* (Chrysomelidae). The roles of water, sugar and nitrogen content have also been implicated as influential in the host selection process (Hanson 1976, Grabstein and Scriber 1982, Scriber 1982). Although some work has already been undertaken to isolate and characterize secondary plant compounds associated with various spurge species and leafy spurge biotypes, effects of these compounds

upon host choices of biocontrol agents like *A. nigriscutis* have yet to be explored. Effects of leafy spurge nutritional characteristics on biocontrol agents have yet to be examined.

Because of the variability demonstrated in feeding preferences of *A. nigriscutis*, certain biotypes of North American leafy spurge may indeed share a greater similarity with some European *Euphorbia* spp. than others. These similarities may be due to relative genetic input from each of the various European *Euphorbia* spp. from multiple introductions (Rowe et al. 1997). Long associations of *A. nigriscutis* with *Euphorbia seguieriana* and *E. cyparissiae* in Europe may have pre-disposed it to prefer plants that share characteristics with these European species. That *A. nigriscutis* demonstrated a consistent preference for some leafy spurge samples supports arguments for taxonomic distinctions being drawn among North American leafy spurges or at least for distinct biotypes to be recognized.

Despite a potential pre-disposition to various leafy spurge biotypes, the importance to the establishment of *A. nigriscutis* of among-site differences in the leafy spurge collected from these Alberta sites is questionable. The overall palatability of leafy spurge at each site was not significantly correlated with *A. nigriscutis* numbers. Among-site differences, though significant, were likely not great enough to influence beetle establishment. Beetles fed upon all of the samples that were tested including the least palatable plant, Cardston (ARC). As *A. nigriscutis* has been reared upon this plant (Alec McClay *pers. comm.*), it is known to be a suitable host for these beetles. This conclusion can be supported

by a lack of significant differences in the numbers of eggs laid on or near any of the samples tested. Although there are differences among these leafy spurge samples, they are all likely acceptable hosts for these beetles.

The variability of leafy spurge at each collection site, however, was correlated with beetle numbers. Sites with greater host variability had larger populations of *A. nigriscutis*. Indeed, the largest sampled *A. nigriscutis* population was on the Edmonton site. This site was also the most variable in terms of leafy spurge biotypes. The least variable site, Millet, was one of three sample sites without beetle establishment.

Why would beetle populations fare better on a site with multiple biotypes? The explanation may lie in the insect's behaviour. These beetles have been shown to cause a highly localised pattern of weed suppression on some sites and have been found in great numbers on the periphery of this roughly circular suppression pattern. Both the pattern of weed suppression and the clumping of beetles upon the solitary wave front, like that described by Kovalev (1990, cited from McClay et al. 1995) for *Zygogramma suturalis* (Chrysomelidae), suggest an aggregated distribution of *A. nigriscutis* in the field.

Host variability on a site has the potential to influence this aggregation behaviour. This selective attraction to and aggregation upon select host plants of the same species on the same site has been demonstrated for the *Eucalyptus stelulata* and *E. pauciflora*-feeding *Chrysophtharta hectica* (Coleoptera: Chrysomelidae) (Strauss and Morrow 1988). *A. nigriscutis* may also be more likely to aggregate upon a preferred host plant. The distribution of feeding

damage in the feeding arenas supports this conclusion. There were significant row and column effects in some replicates of these trials. As damage associated with beetle feeding can be considered indicative of the numbers of beetles upon a plant, these effects were due to the aggregation of beetles upon a preferred, heavily damaged plant and collateral damage to adjacent plants.

Aggregation behaviour has been implicated as a factor that contributes to the success of some biological control agents (Beddington et al. 1978, cited from Begon et al. 1990). An increased level of aggregation may result in an increase in the benefits associated with this distribution - mate location, predator avoidance and an increased ability to overcome host defenses. It may also help to avoid Allee effects (Allee 1931, cited in Begon et al., 1990). Without the influence of multiple biotypes contributing to a clumped distribution, *A. nigriscutis* may be more randomly distributed. As a result, they could be more susceptible to predator pressures or unable to find mates. Beetle distribution in mixed-biotype leafy spurge stands should be examined.

The only other factor examined that was correlated with *A. nigriscutis* numbers was soil pH. These results concur with the findings of McClay et al. (1995). Establishment of *A. nigriscutis* was greatest in areas with more alkaline soil pH. However, sites that are cool or poorly drained may also negatively influence beetle survival. Such sites tend to develop more acid soils or higher levels of organic matter. Thus, this finding does not necessarily mean that the insects respond directly to soil pH or organic matter content (McClay et al. 1995).

Results of these tests suggest that there is cause for the recognition of distinct leafy spurge biotypes but that their differences are not profound enough to deter their use as a host by *A. nigriscutis*. They also offer evidence for the importance of aggregation behaviour, as an explanation of the correlation between within-site variability of leafy spurge and beetle numbers, in the successful establishment of *A. nigriscutis*. What is not clear is the relative importance of within-site variability and site soil pH.

Tables

<i>Nearest town</i>	<i>Maximum number of A. nigriscutis captured in 20 samples</i>	<i>Category</i>
EDMONTON	300	good
CARDSTON †	258	good
FORT MACLEOD	150	good
CLUNEY 1*	133	good
HARDISTY	96	moderate
LETHBRIDGE	72	moderate
PONOKA	10	moderate
CLUNEY 2*	0	poor
MILLET	0	poor
OLDS	0	poor

† These plants were used for leafy spurge biological control agent rearing at, and acquired from ARC. The first *A. nigriscutis* release site in AB. Insects for this release were imported from Hungary. Beetles released at the other sites were collected here and redistributed.

* These sites are ~500m apart but have different levels of beetle establishment.

Table 2-1. Leafy spurge collection sites. Experimental plants were propagated from samples collected from these sites in May of 1998. These sites were revisited in 1999 and ten more samples were taken along a linear transect through each site with the exception of Edmonton and Cardston. These sites were sampled along two linear transects.

CL2	CL1	H	O	M	P	C	L	F	E
P	CL2	C	F	L	M	CL1	O	E	H
E	F	L	CL2	C	H	O	CL1	P	M
C	H	F	M	CL2	CL1	E	P	L	O
CL1	C	E	L	P	CL2	H	M	O	F
L	M	CL2	H	F	O	P	E	C	CL1
O	L	P	C	E	F	M	H	CL1	CL2
M	P	CL1	E	O	L	CL2	F	H	C
F	O	M	CL1	H	E	L	C	CL2	P
H	E	O	P	CL1	C	F	CL2	M	L

Table 2-2. Schematic of Test 1, Replicate 1 to demonstrate Latin square design. Each letter represents a plant sample: C, Cardston (ARC). CL1, Cluney site 1. CL2, Cluney site 2. E, Edmonton. F, Fort Macleod. H, Hardisty. L, Lethbridge. M, Millet. O, Olds. P, Ponoka. Clones of each sample are represented in each row and column only once. Placement of clones was re-randomized in subsequent replicates of this test.

<i>Test</i>	<i>1</i>	<i>2</i>	<i>3</i>	<i>4</i>	<i>5</i>	<i>6</i>	<i>7</i>	<i>8</i>	<i>9</i>
<i>Sample</i>	C(ARC)	O9	F1	C2	CL11	C5	C1	C10	L3
	CL1	P7	F4	C3	CL21	C6	CL19	C7	P4
	CL2	M2	F6	CL13	CL26	C8	CL23	CL18	P5
	E	M3	C4	CL17	E8	CL110	CL27	CL210	P6
	F	M6	CL16	L7	O1	CL14	E9	CL28	C9
	H	M7	CL22	M4	E6	E1	F10	E3	CL12
	L	O4	CL24	O7	H3	E10	M10	E7	E5
	M	O6	H10	P2	H9	E2	P10	H2	H6
	O	F8	L1	P3	H4	E4	H5	H8	H7
	P	F3	L5	P9	M5	H1	O2	L10	P8
<i>Replicates</i>	4	1	1	3	2	4	4	4	4

Table 2-3. Assignment of plants to tests, 1999. Site names are abbreviated: C, Cardston. CL1, Cluney site 1. CL2, Cluney site 2. E, Edmonton. F, Fort Macleod. H, Hardisty. L, Lethbridge. M, Millet. O, Olds. P, Ponoka. The numbers following each abbreviated site name indicate the sample number. For example, CL26 indicates the sixth sample collected along the linear transect through Cluney site 2. The numbers of replicates were limited in some tests by the numbers of plants that could be propagated from each sample.

SNK Grouping	Means of arcsine($\sqrt{(x + 0.5)}/10$) transformed feeding damage ratings	n	Sample
A	0.21	40	Ponoka
AB	0.21	40	Hardisty
ABC	0.20	40	Cluney Site 1
ABC	0.20	40	Lethbridge
ABC	0.19	40	Olds
ABC	0.18	40	Fort Macleod
BCD	0.18	40	Cluney Site 2
CD	0.17	40	Millet
DE	0.15	40	Edmonton
E	0.14	40	Cardston (ARC)

Table 2-4. Results of Feeding Trials 1998. Comparisons of the means of arcsine ($\sqrt{(x + 0.5)}/10$) transformed feeding damage ratings on individual plant samples from ten collection sites. Cardston (ARC) plants were those collected in 1991 and used to rear biological control agents by Alec McClay of the Alberta Research Council. Means were separated with Student-Neuman-Keul's test. There were significant differences in the feeding preferences of *A. nigriscutis* to these plants. Like-lettered groups are not significantly different.

SNK Grouping	Means of arcsine ($\sqrt{(x + 0.5)}/10$) transformed feeding damage ratings	n	Sample
A	0.22	40	Ponoka
AB	0.21	40	Hardisty
AB	0.20	40	Cluney Site 1
ABC	0.20	40	Lethbridge
ABC	0.20	40	Fort Macleod
ABC	0.19	40	Olds
ABC	0.19	40	Cluney Site 2
BCD	0.18	40	Millet
CD	0.17	40	Edmonton
D	0.16	40	Cardston (ARC)

Table 2-5. Feeding Trials, Test 1 1999. Comparisons of the means of arcsine ($\sqrt{(x + 0.5)}/10$) transformed feeding damage ratings on individual plant samples collected in 1998 from ten collection sites. Cardston (ARC) plants were those collected in 1991 and used to rear biological control agents by Alec McClay of the Alberta Research Council. Means were separated with Student-Newman-Keul's test. There were significant differences in the feeding preferences of *A. nigriscutis* to these plants. Like-lettered groups are not significantly different. There were no significant differences between the feeding preferences of *A. nigriscutis* to the plants collected in 1998 when they were tested in both 1998 and 1999.

SNK Grouping	Means of arcsine($\sqrt{(x + 0.5)}/10$) transformed feeding damage ratings	n	Collection Site
A	0.23	350	Hardisty
AB	0.22	390	Cardston
AB	0.22	130	Fort Macleod
AB	0.22	170	Lethbridge
BC	0.22	330	Cluney 1
BC	0.22	260	Cluney 2
BC	0.21	340	Ponoka
BC	0.21	160	Olds
CD	0.21	400	Edmonton
D	0.20	170	Millet

Table 2-6. Feeding Trials 1999. Pooled comparisons, by collection site, of the means of arcsine ($\sqrt{(x + 0.5)}/10$) transformed feeding damage ratings of all of the plants tested in this study. Results of 1999 assessment of 1998 collected plants are included. Means were separated with Student-Newman-Keul's test. There were significant differences in the feeding preferences of *A. nigriscutis* by site. Overall, plants collected from the Hardisty site were the most heavily damaged by *A. nigriscutis* and those from the Millet site were the least. Like-lettered groups are not significantly different.

Collection Site	Sand (%)	Silt (%)	Clay (%)	Soil pH	Organic matter (%)	Weed Density Mean (stems/m ²)	Weed height (cm)	Var	Mean	Beetles
Edmonton	82	14	4	7.7	10	57	60	0.00368	0.21	300
Cardston	74	20	6	7.9	4	180	60	0.00321	0.22	258
Fort	71	19	10	8.1	2	79	40	0.00392	0.22	150
Cluney 1	35	35	30	7.8	7	196	40	0.00317	0.22	133
Hardisty	92	6	2	8.5	3	119	40	0.00278	0.23	96
Lethbridge	67	19	14	7.6	4	115	85	0.00278	0.22	72
Ponoka	80	11	9	6.5	3	66	40	0.00299	0.21	10
Cluney 2	25	29	46	7.6	5	124	50	0.00292	0.22	0
Millet	85	13	2	6.2	7	51	100	0.00274	0.20	0
Olds	42	35	23	7.4	7	No data	70	0.00284	0.21	0

Table 2-7. Examination of some site factors. *Means* indicate the means of arcsine ($\sqrt{(x + 0.5)}/10$) transformed feeding damage ratings for all plants from a particular site. *Var* indicates the variance of the transformed feeding damage ratings for all plants from that site. *Beetles* indicates the greatest number of *A. nigriscutis* captured with twenty sweeps of a net since release of these beetles upon that site. Soil and site plant community data were collected at the time of *A. nigriscutis* release at each site (courtesy, Alec McClay).

Collection Site	Sand (%)	Silt (%)	Clay (%)	Soil pH	Organic matter (%)	Weed Density Mean (stems/m ²)	Weed height (cm)	Var	Mean	Beetles
Edmonton	3	7	8	5	1	8	4	2	9	1
Cardston	5	4	7	3	6	2	4	3	2	2
Fort	6	5	5	2	10	6	7	1	3	3
Cluney 1	9	1	2	4	2	1	7	4	5	4
Hardisty	1	10	9	1	8	4	7	9	1	5
Lethbridge	7	5	4	6	6	5	2	8	4	6
Ponoka	4	9	6	9	8	7	7	5	7	7
Cluney 2	10	3	1	6	5	3	6	6	6	8
Millet	2	8	9	10	2	9	1	10	10	8
Olds	8	1	3	8	2	No data	3	7	8	8

Table 2-8. Rankings of the factors from Table 2-7.

	<i>Sand</i> (%)	<i>Silt</i> (%)	<i>Clay</i> (%)	<i>Soil</i> <i>pH</i>	<i>Organic</i> <i>matter</i> (%)	<i>Weed</i> <i>Density</i> <i>Mean</i> (stems/ m ²)	<i>Weed</i> <i>height</i> (cm)	<i>Var</i>	<i>Mean</i>
<i>r</i>	0.24	-0.08	-0.28	0.71	-0.08	0.17	-0.32	0.73	0.25
<i>p</i>	0.51	0.83	0.44	0.02	0.82	0.67	0.36	0.02	0.48
<i>n</i>	10.00	10.00	10.00	10.00	10.00	9.00	10.00	10.00	10.00

Table 2-9. Results of Spearman rank correlation of site factors with the numbers of beetles per site. Only soil pH and within-site variability in the feeding damage ratings were correlated with beetle numbers (bold). *r* indicates the correlation coefficient, *p* the probability of Ho and *n* the number of samples. *A. nigriscutis* establishment is positively correlated with higher soil pH and within-site variability.

Figures

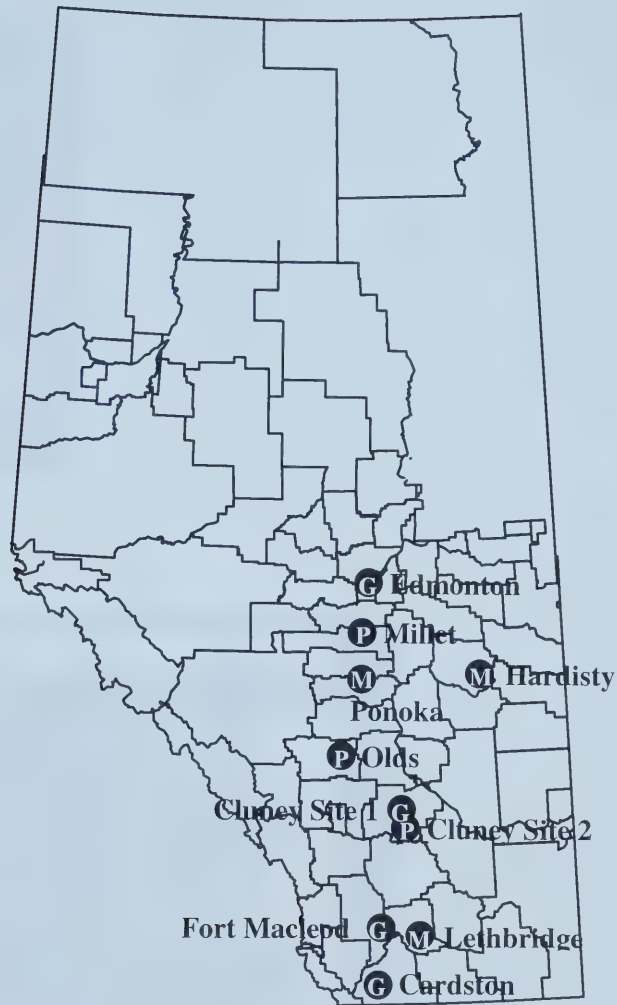


Figure 2-1. Map of Alberta showing the approximate locations of the *A. nigriscutis* releases in or near Edmonton, Ponoka, Hardisty, Olds, Fort Macleod, Lethbridge, Millet, and two sites near Cluney, AB. These releases were categorized into classes based upon the numbers of beetles that were recovered at these sites in the years after release. More than 100, between 10 and 100 and fewer than 10 beetles in 20 sweeps were recovered from good (g), moderate (m) and poor (p) sites, respectively. Plants were collected from these sites in 1998 and 1999. Plants were collected from the Cardston site in 1991 and have since been used to rear biocontrol agents at the Alberta Research Council, Vegreville.



Figure 2-2. Plastic vial containing a rooted leafy spurge cutting.



Figure 2-3. Floor of the feeding trial arena. Ten clones of ten leafy spurge samples were arranged in a randomized Latin square array in the one hundred holes cut in the foam plastic floor of each feeding arena.



Figure 2-4. Feeding arenas 1999.

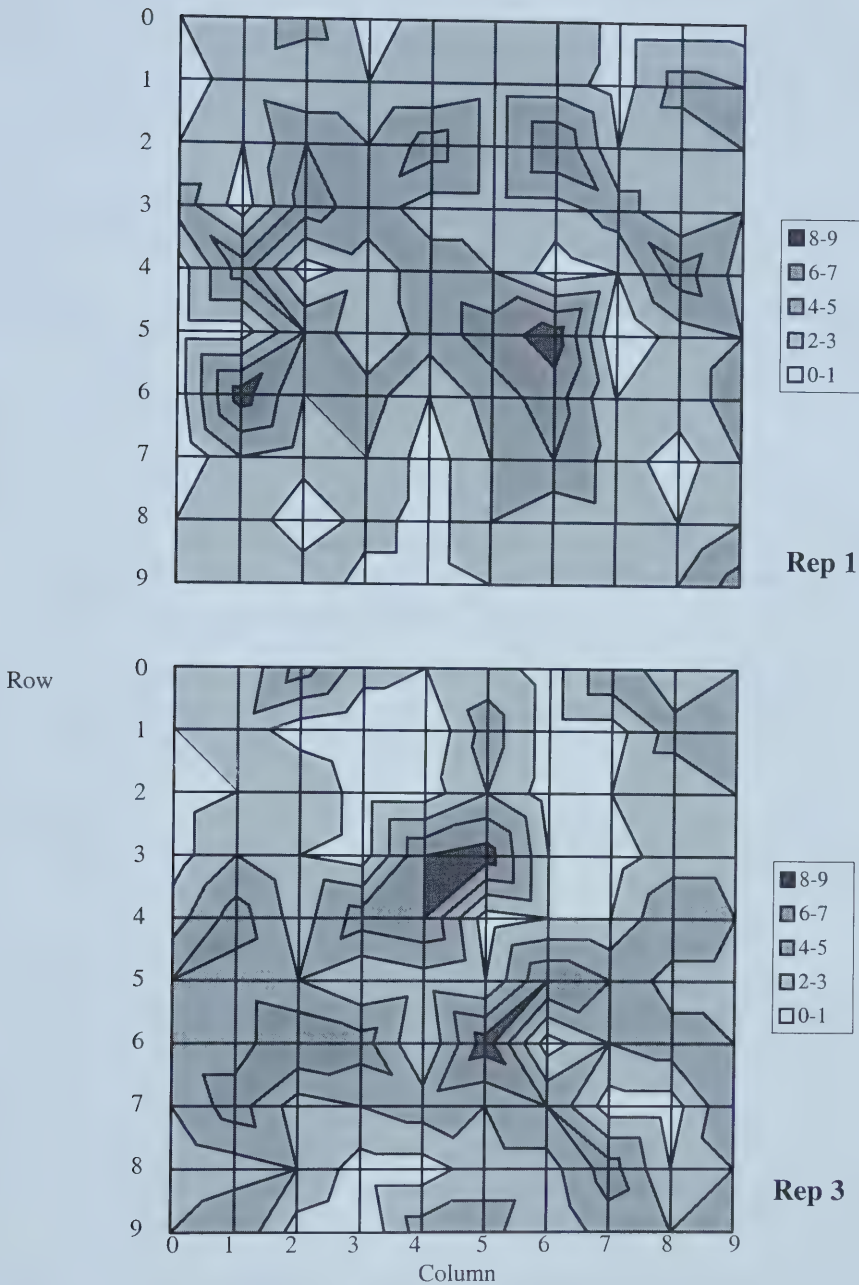


Figure 2-5. Surface plots of the feeding damage ratings for plants collected in 1998 and tested in 1999. Darker colours correspond to greater feeding damage ratings. 0-2 indicates 0 to 20% defoliation and 8-10 indicates 80-100%. There was a significant row effect in the first replicate and significant row and column effects in the third replicate of this test. These effects may be explained by aggregated patterns of feeding damage in both cases. Beetles fed in localized patches within each arena.

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Chapter 3

Volatile cues associated with *Aphthona nigriscutis* congregation behaviour.

Introduction

At some release sites, adult *Aphthona nigriscutis* completely defoliate leafy spurge plants at the original point of release and radiate outward in a roughly circular pattern consistent with the “solitary population wave” described by Kovalev (1990, in McClay et al. 1995) for *Zygogramma suturalis* (Chrysomelidae). Where *A. nigriscutis* populations are large, beetle density is highest and many hundreds of beetles congregate on the wave front (*pers. observ.*). As the beetle population grows the circular pattern expands and the area suppressed increases. This suggests a highly aggregated, well-maintained beetle distribution.

Aggregation behaviour is common among insects and is defined as any movement process that results in a non-uniform, non-random distribution (Southwood 1979). Factors stimulating aggregated distributions may be abiotic, avoidance of harsh or attraction to favourable conditions or biotic, attraction to resource patches or effects of other organisms including conspecifics. When aggregations occur due to effects of conspecifics, Turchin (1998) describes them as congregations.

Congregations result from behavioural responses of organisms to conspecific cues. These cues may be audible, visual or chemical and may act independently or in combination. Many examples of chemically-mediated congregation have been documented in Coleoptera and include beetles in the families Bostrichidae (Fadamiro et al. 1998), Curculionidae (Perez et al. 1997), Scarabaeidae (Yarden and Shani 1994), and Nitidulidae (Petroski and Vaz 1995).

Within Chrysomelidae, members of the subfamily Alticinae, *Phyllotreta cruciferae* (Peng and Weiss 1992) and *Longitarsus jacobaeae* (Zhang and McEvoy 1996) are attracted to conspecific chemical cues, aggregation pheromones, to form congregations. Similar compounds may also influence the congregation behaviour of *A. nigriscutis*.

Host plant volatiles may also play roles in *A. nigriscutis* congregation behaviour. Host plant volatiles are attractive to *Carpophilus hemipterus* (Nitidulidae) (Petroski and Vaz 1995) and *Psylliodes chrysocephala* (Chrysomelidae: Alticinae) (Koritsas et al. 1991), the latter being attracted to mechanically damaged host plants. *Diabrotica* spp. (Chrysomelidae) are also attracted to host plant volatiles, tetracyclic triterpene cucurbitacins. These compounds act as arrestants and feeding stimulants for these cucurbit specialists. Attack and feeding are directly related to the amounts of cucurbitacins B and E (Metcalf et al. 1979).

An interaction between *A. nigriscutis* pheromones and host plant volatiles may also influence beetle behaviour. Although distinctions between a synergy of plant and insect-associated cues and additive effects of these cues are difficult to make, an interaction of pheromones and plant volatiles, where effects of pheromones are enhanced by host plant volatiles, has been described for a number of insect-host systems (for example, Bogner et al.. 1992) and includes beetles in at least four families: Scolytidae (Wood 1982), Scarabaeidae (Yarden and Shani 1994), Nitidulidae (Dowd and Bartelt 1991, Lin et al.. 1992) and Curculionidae (Weissling et al.. 1993).

Suitability of a food source may also influence this behaviour. In a manner similar to *Prostephanus truncatus* (Fadamiro and Wyatt 1996, Fadamiro et al. 1996) that produces a 'suitable resource location and colonisation signal', *A. nigriscutis* may also produce more attractive cues in response to the location or detection of a suitable food source. Whether drawn by conspecific or plant cues or an interaction of these factors, attractive factors apparently influence *A. nigriscutis* behaviour.

Although the frequently observed pattern of circular host plant suppression suggests that aggregated distributions might be relatively stable, congregations are likely not static entities. If conspecifics and/or suitable resources produce attractive cues and conspecifics are within their area of influence, the stimuli that result in congregation should continue to recruit new members. As resources diminish, changes in the composition of these cues could lead to dispersal of superfluous members. Repellence may be a strategy to maintain an appropriate metapopulation density. A metapopulation, as defined by Burkey (1997) is a highly localized group of individual conspecifics resulting from the fragmentation of the population as whole. Groups of *A. nigriscutis* may maintain densities high enough to benefit from aggregated distributions but not so high as to completely deplete resources.

This may be the case with *A. nigriscutis*. Following resource depletion, members of dense beetle populations tend to disperse and colonize new weed stands (Bob Carlson, *pers. comm.*). These new colonies might become sources of immigrants for established colonies as their populations grow and they in turn

disperse. In addition, local extinctions are common among insect species with aggregated distributions (Hanski 1994, Hanski et al. 2000). A congregation of *A. nigriscutis* may suffer some catastrophe, leaving a patch of resources open to re-colonisation. Dispersal from densely populated local patches would encourage re-colonisation of patches where beetle extinction has occurred (Herzig and Root 1996).

Mechanisms of dispersal behaviour, like those of congregation behaviour, are poorly understood for *A. nigriscutis*. High densities may induce production of novel compounds. Compounds associated with congregation might also elicit different responses from conspecifics at different concentrations. Density-dependent production of sex and congregation pheromones has been demonstrated for the southern pine beetle, *Dendroctonus frontalis* (Scolytidae) and attractive compounds elicit dispersal behaviour at higher concentrations (Renwick and Vité 1969).

Roles of each sex in congregation and distribution are also poorly understood. In the case of *D. frontalis*, males select host plants and, through a synergy of pheromones and host plant volatile chemicals, attract females (Wood 1982). Males, by producing another behaviour-modifying compound, repel other males from colonised host plants (Rudinski 1973). Females also produce this compound, though in lesser concentrations. Although repulsive to males at relatively low concentrations, at higher concentrations it is repellent to both sexes and encourages dispersal and colonisation of new host plants (Renwick and Vité 1969).

Field populations of *A. nigriscutis* in North America are as much as 98% female (Hansen 1997). Male-produced aggregation pheromones that are attractive at low concentrations and repel at high concentrations and / or dispersal pheromones that primarily influence males may help to explain these skewed sex ratios in samples of *A. nigriscutis* populations. These effects may also indicate the colonisation and dispersal strategies of *A. nigriscutis* as well as explaining its successful establishment and population growth on some sites despite highly skewed sex ratios.

Field trials were used to describe *A. nigriscutis* distribution shortly after a release and laboratory olfactometer bioassays were used to examine the roles of plant volatiles and pheromones in the congregation and dispersal behaviours of *A. nigriscutis* and assess a potential interaction between plant volatiles and conspecific cues. Olfactometer tests were performed with a range of beetle densities to investigate the production of dispersal pheromones or a concentration effect of aggregation cues. Inter and intra-sex responses were also evaluated at different densities to determine the roles of each sex and the effects of conspecific density on these roles in the production of cues associated with congregation and dispersal. A colonisation and metapopulation dynamic model is also discussed.

Materials and Methods

Beetles. *Aphthona nigriscutis* were collected from a site west of the Beverly Bridge in Edmonton, Alberta in both years of this study (see Chapter 2). This site had seen the release and establishment of *A. nigriscutis* in 1988 and 1989. *A. nigriscutis* were also collected in 2.7 L soil-root samples from this site in early April 1999. Soil samples were placed in pails inside nylon mesh cages in a greenhouse. They were maintained at 24 ± 4 °C and monitored daily until emergence ceased. Numbers and sexes of *A. nigriscutis* were noted throughout the emergence period. Beetles were sexed by examination of the last abdominal sternite (Figure 3-1). In males, this sternite is distinctly lobate and there is a median dark line (LeSage and Paquin 1997). *A. nigriscutis* were maintained on leafy spurge plants in cages in the laboratory at 21 ± 2 °C. *Aphthona lacertosa* were collected with sweep nets in July from a release site on the Stoney Indian Reserve near Morley, Alberta.

Plants. Leafy spurge plants were acquired from the Alberta Research Council (Cardston (ARC) plants) and were propagated in a commercial potting mix (Sunshine Professional) in 5 in round tapered pots. Those intended for beetle maintenance and field assays were grown in 6 in pots. See Chapter 2 for a detailed description of the propagation technique. Plants were maintained in a greenhouse for the duration of the study.

Field Observation Study. This study was conducted near Hardisty, Alberta to assess the distribution of *A. nigriscutis* field populations shortly after release. *A. nigriscutis* were released upon 2 m by 2 m plots in a flat, leafy spurge infested field. Plots were 5 m apart and distinguished from the surrounding area by wooden frames suspended 20 cm above the ground by wooden stakes. Each frame supported a grid of orange twine that subdivided each plot into 36 equal, 33 cm by 33 cm subsections. Movements of *A. nigriscutis* within each plot were assessed by visual counts of their numbers within each subsection.

Counts were conducted daily for the first week and again 21 and 39 days after beetles were introduced to each plot. To determine if beetle spatial distribution was affected by the initial release pattern, one thousand *A. nigriscutis* were released on each plot either as one group in the centre of each plot or uniformly distributed, with 28 beetles in each subsection of each plot. Each type of release and a control plot was replicated four times and arranged according to a randomized block design. There were twelve plots.

To assist with visual counts, beetles were marked shortly before release by placing them into a cloth bag containing approximately 20 ml of a fluorescent powder. The bag was gently turned end over end several times to ensure that all beetles were marked. To evaluate potentially harmful effects of this powder on *A. nigriscutis*, a group of 25 beetles was marked and re-introduced to a laboratory colony. There was no mortality in the treated group up to two weeks after introduction and these beetles were observed feeding on plants and mating both with marked and unmarked beetles.

Each of the release methods was evaluated for patterns of beetle congregation. In addition, percent cover of leafy spurge, grass species, woody species and forbs, all herbaceous plant species excluding leafy spurge, was assessed within plot subsections along a northwest to southeast diagonal of each plot, in early September of 1998 and again a year later.

Laboratory Olfactometer Bioassays. Tests were conducted to assess the attractiveness of plant volatiles and *A. nigriscutis* volatile chemical cues. A no-choice olfactometer (Figure 3-2) was designed for this study. The olfactometer consisted of a cylindrical Plexiglas™ chamber and a glass tube connected by flexible Tygon™ tubing. An electric pump forced air through the chamber and carried volatiles emitted from test materials through a glass tube to its open end. Air passed through an activated charcoal filter to remove volatiles from laboratory air, distilled water to ensure consistent humidity and an airflow regulator before being pumped through the olfactometer. Air flowed through the system at 4 L/minute. This volume of air was determined to produce the greatest movement of beetles up the glass tube. A very slight airflow was perceptible at the open end of the glass tube. The glass tube was marked at 10 cm intervals and suspended on a metal support frame at a 15° angle. This slight upward slope was used to encourage the movement of *A. nigriscutis* into the tube rather than into the laboratory. Between tests, chambers and tubing were cleaned and test materials replaced.

The system differed from 1998 to 1999 by the source of illumination. In 1998, the olfactometer was positioned so that the glass tube sloped upward toward a window and natural light. The olfactometer was also illuminated from above by fluorescent room lighting. As daylight proved to be highly variable at different times of day and from day to day, the olfactometer was illuminated by two 40 watt fluorescent lamps placed 10 cm above the highest point of the glass tube in 1999.

All tests were performed in July and August of both years in the laboratory at 21 ± 2 °C between 0900 and 2000 hr (MST). Individual beetles were introduced to the open end of the glass tube. The end of the tube was covered for 5 seconds and the distance traveled by each beetle in 2 minutes was recorded. Insects that moved out of the apparatus and those that remained stationary were assigned distances of 0. All beetles were randomly chosen from a laboratory colony and were starved for 24 hr before each test unless otherwise specified. Visual counts were performed when plants and beetles were transferred to the apparatus and, in the very rare cases where beetles were lost or died, were replaced. Chambers as well as the glass and connecting tubes were washed with 70% ethanol and distilled water and allowed to dry for a minimum of 30 minutes between tests.

1998. The responses of 10 beetles to each test material were assessed.

Plant volatiles. The attractiveness of volatiles associated with undamaged plants was evaluated. These responses were compared to those of *A. nigriscutis*

to homogenized plants to assess the attractiveness of plant volatiles present in sub-surface tissues. Individual 30 cm plants were ground with a mortar and pestle. Responses of *A. nigriscutis* to these plants were compared to those to an empty chamber and to moistened potted growing mix.

A. nigriscutis feeding upon plants. These tests were conducted to assess the effects of *A. nigriscutis* feeding upon plants and evaluate the effects of conspecific density upon attraction. Groups of 10, 25, 50 and 100 beetles fed upon different leafy spurge plants for 24 hr. These beetles and the plants were transferred to the olfactometer and responses of conspecifics to them were evaluated.

1999: The responses of 40 beetles to each test material were assessed.

Plant volatiles-mechanical damage. The attractiveness of the volatiles associated with mechanically damaged plants was assessed. Using scissors, small pieces were cut from 2, 4, 8 and 16 leaves to simulate increasing levels of feeding damage. Responses of *A. nigriscutis* to these plants were compared to those of *A. nigriscutis* to undamaged plants.

Plant volatiles-feeding damage. The attractiveness of the plant volatiles associated with beetle feeding was examined. Different types of insect feeding and mechanical damage have been shown to influence volatiles released by plants (Takabayashi et al.. 1998, Turlings et al. 1998). In order to investigate the

potential role of plant volatiles produced in response to beetle feeding, feeding damage had to be simulated without the influence of beetle-produced stimuli. Insects can release chemical cues in a number of ways including frass (Peng and Weiss 1992), oral exudates (Poirier and Borden 1995) and specialised glands (Schindek and Hilker 1996). As it had yet to be determined how or if *A. nigriscutis* produces the chemical cues associated with congregation behaviour, *A. nigriscutis* and their potentially attractive influence had to be excluded. Another flea beetle, *Aphthona lacertosa*, shares a number of similarities with *A. nigriscutis* including size and host preference (*Euphorbia* spp.) so was chosen to simulate *A. nigriscutis* feeding.

These two species segregate when released on the same site (D. C. Thompson, *pers. comm.*), so factors that influence the distribution of one species likely do not influence that of the other. These factors may include attractive chemical cues. If chemical cues produced by one beetle species are not attractive to the other, the effects of plant volatiles produced in response to or released by *A. lacertosa* feeding on *A. nigriscutis* could be examined without the influence of cues produced by *A. nigriscutis*. In this manner, the effects of volatiles released by beetle feeding may be examined without the influence of conspecific beetle cues.

Groups of 20 *A. lacertosa* fed upon plants for 24 hr. In one test, beetles remained upon the plants. In another, beetles were removed but their frass, a possible source of volatile cues, was left on the plants. The attractiveness of both groups to *A. nigriscutis* was compared to that of undamaged plants.

Conspecific cues- A. nigriscutis on plants. Groups of 20, 40, 60, 80, 100 and 120 *A. nigriscutis* fed upon leafy spurge plants for 24 hr prior to the test. These plants and beetles were transferred to the olfactometer and the responses of *A. nigriscutis* to them were compared to those of *A. nigriscutis* to undamaged plants.

Conspecific cues- A. nigriscutis alone. This test was performed to determine if *A. nigriscutis* responses were dependent upon the presence of plants or were subject entirely to the densities of conspecifics. Responses of *A. nigriscutis* to groups of 20, 40, 60, 80, 100 and 120 conspecifics in an otherwise empty chamber were assessed and compared to empty chambers and to same-size groups feeding on plants. Test material beetles were allowed to feed upon leafy spurge plants for 24 hr prior to introduction to the apparatus.

Effects of sex and density. Intra- and inter-sex attraction was tested at both low and high densities. Groups of 20 and 120 *A. nigriscutis* were found to be significantly more and less attractive, respectively, than undamaged plants in a previous test so these densities were chosen to examine effects of sex and density on congregation. Groups of 20 and 120 *A. nigriscutis* of each sex fed separately on plants for 24 hr prior to the test. Responses of each sex to these groups were assessed and compared to the attractiveness of undamaged plants to both sexes.

Frass as a source of conspecific cues. To determine if conspecific compounds that influenced *A. nigriscutis* responses were secreted in frass, a 15 cm diameter filter paper was fitted around the base of a plant. The paper was cut down its radius to allow the plant's stem to be positioned in its centre. Groups of 20 or 120 males or females fed upon these plants for 24 hr. Beetles were removed and the filter paper, which collected frass dropped by *A. nigriscutis*, was placed into another, empty chamber. The attractiveness of this filter paper was compared to that of an untreated filter paper.

Vial Test. This test was conducted in late May, as *A. nigriscutis* emerged from soil samples and again in late August of 1999 with net-collected beetles to distinguish the effects of volatile chemical cues produced by each beetle sex and to assess effects of beetle age on the production of attractive cues. Each *A. nigriscutis* was sexed (LeSage and Paquin 1997) and sealed inside a 1.5 by 6 cm glass vial containing a 2 by 6 cm piece of filter paper. The paper conformed to the shape of the vial so that approximately half of the vial's inner surface was covered. After 24 hr the beetle was removed. Another *A. nigriscutis* was introduced and the vial's open end was connected to the open end of another vial containing an untreated piece of filter paper (Figure 3-3). This beetle was allowed 30 minutes to acclimate. Its position, in one vial or the other was then recorded as binomial data at 15 min intervals for 3 hr. A beetle located on the treated side was scored as '1'. There was a minimum of 30 replicates for each of the four variations of the experiment in both May and August. Both newly emerged and

net-collected *A. nigriscutis* fed on plants in the laboratory for a minimum of 24 hr before each test.

Field Trapping Experiment. The attractiveness of some of the test materials examined in the olfactometer experiment was assessed in the field. Tests were conducted along the bottom of a hill parallel to the Beverly Bridge release sites in Edmonton, Alberta. Test plants for the study were grown to heights of 40 to 50 cm, under the same conditions and in the same growing mix as those propagated for the olfactometer tests.

An array of soapy-water traps was used to capture adult *A. nigriscutis*. Each trap consisted of a pot placed on top of an upside-down 12 by 6 cm plastic tray placed in the centre of a 60 cm diameter by 12 cm deep plastic tray (Figure 3-4). The larger trays were filled with soapy water to a depth of 6 cm. Test materials were covered with a fine cloth mesh bag to allow volatiles out, retain test insects and exclude field insects. Bags were secured to pots with elastic bands.

Three tests were conducted. Each compared the numbers of males and females and total numbers of *A. nigriscutis* captured in traps baited with different test materials. In the first test, a potted plant, a pot of soil mix, a mechanically damaged plant and two plants that groups of *A. nigriscutis* had fed upon for 24 hr were used as bait. *A. nigriscutis* but not their frass were removed from one beetle-infested plant approximately 30 minutes before this test. Plants were

mechanically damaged by cutting small pieces from sixteen leaves just prior to each test.

For the second test, groups of 20 *Aphthona lacertosa* fed upon two plants for 24 hr. These beetles, but not their frass, were removed from one plant approximately 30 minutes before the test. The numbers of *A. nigriscutis* captured with these baits were compared to those captured by traps with 20 *A. nigriscutis* feeding upon a plant, moistened, potted soil mix and an undamaged plant.

In the third test, the numbers of *A. nigriscutis* captured by traps baited with 20 male *A. nigriscutis* on plants, 20 female *A. nigriscutis* on plants, moistened, potted soil and an undamaged plant were compared.

In all three tests, baits were replicated four times and arranged in a randomized block design. Pots were arranged in a line, 5 m apart and approximately 2 m below a dense strip of leafy spurge at the mowed bottom of the hill (Figure 3-5). Traps were monitored after 48 hr.

Data Analysis.

Field Observation Study. For the Hardisty field releases, Iwao's (1968) regression method was used to assess and compare beetle distributions on each release type at each time interval. Iwao's method involves calculation of the regression of Lloyd's (1967) "mean crowding", m^* , to the population mean, m . Lloyd's "mean crowding" is derived as follows:

$$m^* = m + (\sigma^2 / m - 1),$$

where m represents the population mean and σ^2 the variance. Iwao discovered that m^* has a linear relationship with m (Iwao 1968, cited from Christensen et al. 1977).

$$m^* = \alpha + \beta m$$

where α and β are constants that represent theoretical distributions. For example, $\alpha = 0$ and $\beta = 1$ for the Poisson distribution. The value of α , the Y-intercept, indicates the number of individuals that comprise the basic component of the distribution. This value is called the index of basic contagion (Christensen et al. 1977). Values of $\alpha > 0$ indicate that the basic component is a group of individuals, $\alpha = 0$ indicates this component is the individual and $\alpha < 0$ indicates repulsion (Southwood 1986). The regression coefficient β , the density contagiousness coefficient, indicates the distribution. Values of $\beta > 1$ indicate aggregation, $\beta = 1$ indicates Poisson distribution and $\beta < 1$ indicates uniform distribution (Christensen et al. 1977). As 95% confidence intervals were calculated for regression coefficients and Y-intercepts of each function, comparisons between the distributions of beetles on each release type at each monitoring time could be made and each could be compared to the Poisson distribution. The values m , m^* and σ^2 were used in these calculations as counts represented the entire beetle population at this site. For the purpose of calculation, each plot was considered to be composed of six N-S running columns. Each column contained six plot subsections. All calculations (mean, variance and well as Lloyd's mean crowding coefficient) were made using the numbers of *A. nigriscutis* per plot subsection per monitoring date. The numbers of *A. nigriscutis* per plot and the cover of each plant type were also compared by ANOVA.

Laboratory Olfactometer Bioassays. Results of the olfactometer bioassays in both years were transformed by $\sqrt{x+0.5}$ to stabilize the variance and treatments compared by ANOVA. Means were separated by Student-Newman-Keul's test.

The binomial data produced at each time interval of the vial test was tested against the null hypothesis that 50% of *A. nigriscutis* were expected for each of the two choices by testing the fit of the data to the binomial distribution using a *Vassarstats* computer program (Lowry 2000). The program, using the following formula, tested the probability of fit to the binomial distribution.

$$P_{(k \text{ out of } n)} = \frac{n!}{k!(n-k)!} (p^k)(q^{n-k})$$

Where n is the numbers of beetles tested, p is the expected probability of a beetle on the treated side, 0.5 for these tests, k is the number of beetles on the treated side and q is the expected probability that no beetles will be on the treated side, again 0.5.

Field Trapping Test. The field trapping tests were analyzed by ANOVA. All statistical tests were at the 5% level of significance.

Results

Beetles. Adult *A. nigriscutis* began to emerge from the soil samples April 26 1999 and continued until May 16 1999. Of these 191 of 255 (74.9%) were female (Figure 3-6).

Field Observation Study. Comparisons of the slopes and intercepts of Iwao's patchiness regression for central and uniform groups at each monitoring time demonstrated that *A. nigriscutis* formed congregations on plots with both release types within one day of release. Although *A. nigriscutis* were aggregated on both release types 24 hr after beetles were released, they were significantly more aggregated on the central releases until the fourth day (Figure 3-7). Beyond this time, there were no significant differences in the levels of aggregation (Figure 3-8).

Overlap of the 95% confidence interval of the slope, β , the "density contagiousness coefficient", with a value of 1, indicating a random distribution, was evident for the function associated with central releases at 21 days after release and with that associated with uniform releases 39 days after release. There were, however, no significant differences among the slopes of the functions associated with either central or uniform releases, indicating no difference in the levels of aggregation, at either of these monitoring times (Figures 3-9, summarized in Figure 3-10).

The 95% confidence interval of the intercept α , the basic component of the distribution overlapped a value of 0 at most time intervals. On those plots

with a central beetle release at 21 days after release, α was significantly lower than 0 and the 95% confidence interval of β overlapped a value of 1 indicating both a Poisson distribution and repulsion on these plots at this time, offering no clear indication of the numbers of beetles per congregation (Figure 3-11).

Examination of the distribution of beetles over the plots at each monitoring date suggests that beetle congregations were ephemeral. There appears to have been a great deal of exchange between relatively small groups, with group size and location shifting constantly (Figures 3-12 and 3-13).

Examination of numbers of *A. nigriscutis* per plot revealed significant differences both by release type and monitoring date ($p = 0.0434$ and 0.0001 , respectively) but no block effect ($p = 0.6985$) or interaction between factors ($p = 0.4061$). More beetles were found on the central release plots than those with uniform releases. Mean *A. nigriscutis* numbers on these release types were 189.3 and 168.9, respectively. The numbers of *A. nigriscutis* diminished significantly from the time of release to the first monitoring date, again by the third date and yet again by the sixth (Table 3-1).

There were no differences in the cover of leafy spurge ($p = 0.9249$), grass ($p = 0.9742$), forbs ($p = 0.2992$) or woody plant species ($p = 0.9873$) between central or uniform releases and check plots in 1998. There was a significant block effect for grass cover ($p = 0.0006$) in 1998 with significantly more grass found in block 1. There were no differences in the cover of leafy spurge ($p = 0.3761$), grass ($p = 0.1489$), forbs ($p = 0.3416$) or woody plant species ($p = 0.8193$) between central or uniform releases and check plots in 1999. Again, there was a

significant block effect for grass cover ($p = 0.0001$) in 1999 with more grass in block 1 than 2 and more in block 2 than in 3 or 4.

Laboratory Olfactometer Bioassays.

1998. *Plant volatiles.* Comparisons of beetle responses revealed no significant differences ($p = 0.40$) in the attractiveness of an empty chamber, soil, homogenized plants or undamaged plants.

A. nigriscutis feeding upon plants. There were significant differences in the responses of *A. nigriscutis* to increasing numbers of conspecifics on plants ($p = 0.0103$). *A. nigriscutis* were significantly more attracted to plants with 25 beetles than to plants with 10 or 100 beetles (Table 3-2).

1999. *Plant volatiles-mechanical damage.* *A. nigriscutis* demonstrated no significant preference to any of the numbers of damaged leaves tested ($p = 0.1139$).

Plant volatiles- feeding damage. *A. nigriscutis* showed no preference to plants exposed to the feeding of 20 *A. lacertosa* and this beetle's frass or to 20 *A. lacertosa* adults feeding upon plants ($p = 0.2968$).

Conspecific cues- A. nigriscutis on plants. As in 1998, there were significant differences in the responses of *A. nigriscutis* to increasing numbers of

conspecifics on plants tested in 1999 ($p = 0.0001$). Means separation by SNK revealed that beetles were significantly more attracted to 20 conspecifics than to any other sized group and significantly less attracted to 120 conspecifics than to all but 100 and 80 conspecifics (Table 3-3). Beetles were highly attracted to relatively small groups and attraction fell off with increasing numbers (Figure 3-14).

Conspecific cues-A. nigriscutis alone. Significant differences in the responses of *A. nigriscutis* to different numbers of conspecifics were again evident in this test ($p = 0.0001$). Means separation by SNK showed that groups of 20 *A. nigriscutis* were significantly more attractive than groups of 120 conspecifics (Table 3-4). There were no significant differences between the responses of *A. nigriscutis* to an empty chamber and either 20 or 120 conspecifics.

Comparisons of the responses of *A. nigriscutis* to groups of conspecifics with and without plants, and means separation using SNK, revealed the beetles were significantly more attracted to conspecifics on plants than those without plants. A significant ($p = 0.0280$) effect of substrate, the presence or absence of plants indicated an effect of plants on beetle response.

Effects of sex and density. The responses of male and female *A. nigriscutis* to undamaged plants revealed not significantly different ($p = 0.2129$). There were no significant differences in the responses of female beetles to 20 or 120 conspecific females feeding upon plants or undamaged plants ($p = 0.7525$).

There were, however, significant differences ($p = 0.0001$) in the responses of male *A. nigriscutis* to females. Males were less attracted to 120 than to 20 female *A. nigriscutis* or to undamaged plants (Table 3-5). There were also significant differences in the responses of both female and male *A. nigriscutis* to males on plants ($p = 0.0001$ for both). Female beetles were more attracted to 20 males. There were no significant differences in the attraction of female *A. nigriscutis* to either 120 males or an undamaged plant (Table 3-6). Male *A. nigriscutis* were more attracted to 20 and less attracted to 120 males than an undamaged plant (Table 3-7).

Responses of female and male beetles to male conspecifics on plants were also compared. There were significant differences ($p = 0.0001$) in the overall response of females and males. Females traveled greater distances up the olfactometer tube toward male *A. nigriscutis* on plants than did males. There was also a significant interaction of the factors beetle sex and beetle numbers ($p = 0.0004$). Male and female *A. nigriscutis* responded to male conspecifics in significantly different manners at these densities. Males were more sensitive to repellent cues and females were more sensitive to attractive cues.

Frass as a source of conspecific cues. There were significant differences in responses of female *A. nigriscutis* to frass of conspecific males ($p = 0.0044$). Frass of male beetles was significantly more attractive to female beetles at densities of both 20 and 120 beetles per plant than untreated filter paper (Table 3-8). Frass of male beetles was also significantly ($p = 0.0001$) more attractive to

male beetles at densities of 120 beetles per plant than at 20 beetles per plant or to untreated filter paper (Table3-9). There were no significant differences in the responses of male beetles to 120 or 20 female beetles per plant, or to untreated filter paper ($p = 0.1340$). Frass of female *A. nigriscutis*, despite the negative influence of high densities of females on plants to conspecific males, did not influence their behaviour ($p = 0.1340$) (Table3-10).

Vial Test.

In the spring of 1999, with the exception of the seventh time interval, when females were repelled by males, *A. nigriscutis* were evenly distributed between treated and untreated filter papers (Table 3-11).

In the summer of 1999, females were attracted to male-associated cues at all time intervals and to females at the eighth (Table 3-12). Males were not attractive to other males nor were females, for the most part, attractive to either sex in this test.

Field Trapping Experiment.

Test 1. There were significant differences in the total numbers of *A. nigriscutis* captured with these baits ($p = 0.0001$). Significantly more *A. nigriscutis* were captured in traps baited with twenty randomly chosen, mixed-sex conspecifics feeding upon a plant than by traps with any other bait. *A. nigriscutis* frass on plants was also more attractive than undamaged plants or potted soil but

not significantly more attractive than mechanically damaged plants (Table 3-13). Significant differences in the numbers of male ($p = 0.0001$) and female ($p = 0.0028$) *A. nigriscutis* captured in these traps were also evident. Significantly more males and females were captured with twenty conspecifics than with any other bait (Tables 3-14 and 3-15). There were no significant block effects for the total numbers, or numbers of males or females captured ($p = 0.0594, 0.4971$ and 0.1044 , respectively).

Test 2. There were no significant differences in the total numbers of *A. nigriscutis* ($p = 0.0604$) (Table 3-16) or numbers of female *A. nigriscutis* captured ($p = 0.2424$) (Table 3-17). However, there were significantly more male *A. nigriscutis* captured with twenty conspecifics on plants than with the other baits ($p = 0.0151$) (Table 3-18). There were also significant block effects for the numbers of females and total numbers of *A. nigriscutis* captured ($p = 0.0040$ and 0.0204) but no such effect for the numbers of males captured ($p = 0.7703$). The results of this test may have been influenced by very cool, windy conditions when the test was run. The crucifer flea beetle, *Phyllotreta cruciferae* requires a minimum temperature of 14°C for flight (Lamb 1983). Temperature may also affect *A. nigriscutis* flight and mobility.

Test 3. Significantly more *A. nigriscutis* were captured in traps baited with male conspecifics feeding upon a plant than those baited with females on a plant, undamaged plants or soil ($p = 0.0006$) (Table 3-19). There were significant

differences in the numbers of females, but not of males, captured with each type of bait ($p = 0.0419$ and 0.1133 , respectively) (Tables 3-20 and 3-21). There were significantly more female *A. nigriscutis* captured with males as bait than with potted soil. There were also significant block effects for numbers of males, females and total numbers of *A. nigriscutis* captured (males: $p = 0.0031$, females: $p = 0.0375$, total: $p = 0.0001$).

Discussion

Adult *Aphthona nigriscutis* assume aggregated distributions in the field. Immediately after most *A. nigriscutis* dispersed from the plots or were lost to predators, the remaining beetles formed congregations on uniform plots and maintained them on central release plots. Aggregation remained relatively constant on the central releases but was in a state of immediate and constant flux on uniform releases. Those factors responsible for formation of congregations were favoured by a central release pattern. There is also the possibility that congregations of *A. nigriscutis* on central releases may have influenced the distribution of conspecifics on nearby uniform plots. This will be discussed in a later section.

The stability of congregations on central release plots was not maintained for long. Congregations were dynamic. New groups appeared and disappeared, presumably establishing new congregations and exchanging individuals between groups, on uniform plots immediately after release and within four days of release on central plots without an obvious pattern.

Volatiles from undamaged or heavily mechanically damaged plants generally did not influence the responses of *A. nigriscutis*, nor did volatiles produced or released by Cardston (ARC) plants when fed upon by *A. lacertosa* in either laboratory or field trials. That volatile chemicals from undamaged, mechanically or *A. lacertosa* feeding-damaged plants had little effect on *A. nigriscutis* responses in these tests suggests that these compounds are either of

little importance in congregation behaviour, were not present in concentrations high enough to elicit a response or do not affect close proximity aggregation.

Unlike volatiles associated with Cardston (ARC) plants, conspecific-produced volatile chemical cues were shown to significantly influence *A. nigriscutis* behaviour. They also elicit significantly different responses at different conspecific densities. *A. nigriscutis* were highly attracted to relatively low densities of conspecifics in both the olfactometer and the field trapping tests. This attraction fell off gradually until a repellent effect was apparent at higher densities in olfactometer tests. Conspecific cues contribute to both congregation and dispersal behaviour in *A. nigriscutis*. However, as there were significant differences in the responses of *A. nigriscutis* to same-size groups of conspecifics with or without plants, the effects of plants cannot be discounted. Peng and Weiss (1992) demonstrated that *P. cruciferae* requires contact with or feeding upon a suitable host plant for production of its aggregation pheromone.

A. nigriscutis, like *Prostephanus truncatus* (Fadamiro and Wyatt 1996) that produce a 'suitable resource location and colonisation signal', may produce more attractive cues in response to the presence of a suitable food source. This effect may also indicate the role of a plant-produced pheromone constituent, as demonstrated for *Scolytus multistriatus* (Scolytidae) (Gore et al. 1977, cited from Lanier 1983), or a pheromone precursor, as demonstrated for *Ips paraconfusus* (Coleoptera: Scolytidae) (Hughes 1974).

Congregation and dispersal cues are not exerted equally by or on both sexes at all densities. The interaction of the sexes is consistent with that

demonstrated for *Dendroctonus frontalis* (Renwick and Vité 1969). At relatively low *A. nigriscutis* densities, male beetles produce chemical cues that attract both sexes. Females respond more positively to these cues. These conclusions were supported by the results of the field trapping tests. More female beetles were captured with traps baited with males than with those baited with females or undamaged plants. A lack of significant differences in the numbers of males captured with these baits indicates a lower level of attractiveness of male *A. nigriscutis* to other males.

As the densities of male *A. nigriscutis* increased in olfactometer tests, the responses of both sexes to these cues diminished. A repellent effect of male *A. nigriscutis* on conspecific males was apparent at densities of 120 per plant. Responses of female *A. nigriscutis* to groups of 120 males were significantly less than those to plants with 20 conspecific males though not less than to undamaged plants. If the apparent trend of reduced attraction to increasing densities prevailed, male *A. nigriscutis* would have become repulsive to conspecific females at yet higher densities (>120).

High densities of females also repel males. Males produce cues associated with the attraction, at low densities, of males and females and repellence, at high densities, of both males and likely females. Female *A. nigriscutis* produce repellent cues at high densities and these affect only males.

Field trapping tests also showed the attractiveness of the frass of mixed-sex groups of *A. nigriscutis*, albeit on plants, to both sexes. Examination of the responses of males and females to the frass of either sex with the olfactometer

illustrated that congregation and dispersal behaviours are likely due to the production of two distinct compounds or groups of compounds, one associated with the formation of congregations, one with dispersal. Both male and female *A. nigriscutis* responded positively to the frass of males, the sex shown to be associated with the production of attractive cues, at all male densities tested. The attraction of males was greatest to frass of conspecific males at the highest density. The attraction of females to male frass was most pronounced at 20 males per plant and was maintained at higher densities. No repellent effect was evident in the responses of males or females to male-produced frass. Neither was there a repellent effect of the frass of females at high densities on males. Attractive cues are secreted in the frass of males and these cues influence the behaviours of both males and females. Repellent cues are produced in another manner and are able to overcome the attractive effect of these cues at high densities.

An asymmetrical response was also demonstrated in the vial tests; only females responded positively to male-associated cues. Neither sex responded consistently to female-associated cues. Because responses to individual beetles were tested, these responses should be considered analogous to the responses of *A. nigriscutis* to low densities of conspecifics in the olfactometer tests. These results are consistent with those of the olfactometer and field-trapping tests, in which females were significantly more attracted than males to male-produced congregation cues and neither males nor females responded positively to female-associated cues. Also, as these results were obtained without the influence of

plant volatiles, conspecific cues are likely the primary stimuli for close-proximity congregation behaviour on this leafy spurge biotype.

The effects of this compound(s), however, were not evident when newly emerged beetles were tested. Dietary precursors to pheromones are required for at least one other insect species, *Bactrocera papayae* (Hee and Tan 1998).

Although this conclusion is strictly speculative, the 24 hr immediately following emergence that *A. nigriscutis* was allowed to feed upon plants may not have been time enough to metabolize dietary precursors and produce pheromones. The possibility of temporal strategic differences, shortly after emergence or later in the season, in the production of congregative cues in this beetle cannot be ruled out.

Male produced aggregation pheromones are not uncommon in Coleoptera and have been demonstrated for beetles in Scolytidae (Wood 1982), Nitidulidae (Bartelt and Dowd 1991), and Curculionidae (Weissling et al.. 1993). Although evidence for asymmetrical responses of sexes exists for members of the Alticinae (Zhang and McEvoy 1994) there are no records as yet of a male-produced congregation pheromone for this group.

A. nigriscutis that emerged from soil cores collected from the Edmonton site were approximately 75% female. Disproportionate attraction of female beetles to congregations would further skew local sex ratios, thus, collection sex ratios may not necessarily reflect those of an entire population. The unequal sex ratio of *A. nigriscutis* does not, however appear to impede local population growth. At the Beverly Bridge release site, initial populations of hundreds of beetles grew to populations of hundreds of thousands within a few years

(Stromme et al. 2000). This rapid population growth in spite of a disparity of males suggests that *A. nigriscutis* may employ a polygamous mating strategy, with individual males mating with and fertilizing the eggs of multiple females.

Collectively, along with the results of the Hardisty field monitoring experiment, that demonstrated rapid, sustained initiation of congregation behaviour and the formation of ephemeral congregations, these results suggest a potential resource patch colonisation and metapopulation maintenance strategy. A metapopulation, as defined by Burkey (1997) is a highly localized group of individual conspecifics resulting from the fragmentation of the population as whole. An important point of consideration is the scales of both fragmentation and resource patches. For the purpose of the proposed model, individual or very small groups of plants comprise the resource patch to be colonised and thus the area occupied by fragments of the beetle population. Each congregation is a metapopulation.

The scale of influence of conspecific cues must also be considered, as must the presence of conspecifics within the area of influence. The olfactometer designed for this study was capable of measuring only relatively close-proximity responses and behaviours as were the two field studies. Extrapolations of these results and interpretations to attempt to explain long-distance colonisation strategies would be inappropriate.

Proposed Metapopulation Maintenance Model

Males are the initial colonisers of resource patches and, at relatively low densities, produce chemical cues that draw conspecifics of both sexes to the patch. Female numbers would increase more rapidly than those of males as they are disproportionately more attracted by male cues. At relatively high densities, possibly evolved to coincide with the density at which the integrity of a resource patch is threatened, both males and females produce cues that repel male beetles. Although female beetles are also repelled by male-associated cues at high densities, males, due to combined effects of male and female-associated dispersal cues and their lower dispersal threshold in response to male-associated cues, would be subject to greater dispersal pressure. An increased concentration of male produced congregation cues, an increased number of collisions or contact with conspecifics, depletion of resources, or another factor altogether may stimulate the production of repulsive cues by both males and females.

Exiled males may, should additional resource patches be available, attempt to establish their own colonies nearby and attract potential mates and additional males. As these metapopulations grew in size, beetles would again be stimulated to disperse. Recruitment and dispersal may be cyclical, dependent upon individual metapopulation density and may also involve the competition between groups for members. Newly colonised patches would be less attractive than those with slightly larger numbers of attractive cue producers.

This model may explain the larger numbers of beetles seen on the Hardisty plots with central releases of beetles. Those groups of *A. nigriscutis* released in

the centres of plots, although initially losing a large number of members to dispersal and predation by ants, spiders and others, as did those released uniformly over the plots, retained more members than uniformly distributed beetles. After the initial repellence from the very dense metapopulation, smaller metapopulations were established. The combined attractive influence of these larger, more aggregated groups may have drawn beetles from the uniform plots or helped to prevent dispersal from the area immediately about these groups. The males in a large group, adjacent to and large enough to overwhelm the attractive cues produced by small groups, but not so large as to repel its own members, may also be able to better monopolize local females. These groups, however, would also be attractive to males and may annex nearby, less attractive groups, increasing metapopulation size and driving the dynamic towards repellence. As demonstrated with the Hardisty field observations, the cycle of growth and dispersal of metapopulations may take only hours.

Recruitment and dispersal may also depend on resource integrity and distribution. Immigration and emigration rates within each metapopulation could cycle about an equilibrium point as long as resources were available. The effects of resource depletion on attractive and repellent effects should also be examined.

Compounds that influence congregation and dispersal behaviour should be isolated and tested individually to assess their effects. In addition, the factors that initially draw beetles to resource patches remain unclear.

Tables

<i>SNK Grouping</i>	<i>Mean</i>	<i>n</i>	<i>Days After Release</i>
A	1000	8	0
B	156.75	8	1
B	149.88	8	2
C	97.38	8	3
CD	83.75	8	4
CD	75.75	8	5
DE	39.63	8	6
E	4.88	8	21
E	3.88	8	39

Table 3-1. 1998 Hardisty Field Test. The mean numbers of beetles per plot separated by Student-Newman-Keul’s test. Beetles were released on plots in groups of one thousand either in the centre of each plot or uniformly distributed over it. There were significant differences in the numbers of beetles per plot on both release types by monitoring date. Although large numbers of beetles were lost to predators, many may have dispersed from the plots as well.

<i>SNK Grouping</i>	<i>Means of $\sqrt{(x+0.5)}$ transformed responses</i>	<i>n</i>	<i>Beetles</i>
A	6.35	10	25
AB	4.71	10	0
AB	4.46	10	50
B	3.68	20	10
B	3.46	10	100

Table 3-2. 1998 Olfactometer tests. Means separation of the $\sqrt{(x+0.5)}$ transformed responses of *A. nigriscutis* to groups of 0, 10, 25, 50, and 100 conspecifics on plants by Student-Newman-Keul's test. There are no significant differences between like-lettered groups. Moderate densities of beetles were more attractive than undamaged plants or the other group sizes tested.

<i>SNK Grouping</i>	<i>Means of $\sqrt[3]{(x+0.5)}$ transformed responses</i>	<i>n</i>	<i>Beetles</i>
A	5.21	40	20
B	4.28	40	40
BC	4.09	40	60
BC	3.89	40	0
CD	3.42	40	100
CD	3.37	40	80
D	2.78	40	120

Table 3-3. 1999 Olfactometer tests. Means separation of the $\sqrt[3]{(x+0.5)}$ transformed responses of *A. nigriscutis* to groups of 0, 20, 40, 60, 80, 100 and 120 conspecifics on plants by Student-Newman-Keul's test. There are no significant differences between like-lettered groups. Moderate densities were again the most attractive and high densities were repellent.

<i>SNK Grouping</i>	<i>Means of $\sqrt[3]{(x+0.5)}$ transformed responses</i>	<i>n</i>	<i>Beetles</i>
A	4.29	40	20
BA	3.88	40	40
BAC	3.46	40	0
BAC	3.40	40	80
BC	3.23	40	60
BC	3.16	40	100
C	2.57	40	120

Table 3-4. 1999 Olfactometer tests. Means separation of the $\sqrt[3]{(x+0.5)}$ transformed responses of *A. nigriscutis* to groups of 0, 20, 40, 60, 80, 100 and 120 conspecifics without plants by Student-Newman-Keul's test. There are no significant differences between like-lettered groups. Although moderate densities were again the most attractive, there were significant differences in the responses of beetles to conspecifics with and without plants. Beetles on plants were more attractive.

<i>SNK Grouping</i>	<i>Means of $\sqrt[3]{(x+0.5)}$ transformed responses</i>	<i>n</i>	<i>Beetles</i>
A	3.34	40	20
A	3.30	40	0
B	2.00	40	120

Table 3-5. 1999 Olfactometer tests. Means separation of the $\sqrt[3]{(x+0.5)}$ transformed responses of male beetles to groups of 0, 20 and 120 female beetles on plants by Student-Newman-Keul's test. There are no significant differences between like-lettered groups. High densities of females repelled males.

<i>SNK Grouping</i>	<i>Means of $\sqrt[3]{(x+0.5)}$ transformed responses</i>	<i>n</i>	<i>Beetles</i>
A	4.79	40	20
B	3.07	40	120
B	3.04	40	0

Table 3-6. 1999 Olfactometer tests. Means separation of the $\sqrt[3]{(x+0.5)}$ transformed responses of female beetles to groups of 0, 20 and 120 male beetles on plants by Student-Newman-Keul's test. There are no significant differences between like-lettered groups. Moderate densities of males were very attractive to females.

<i>SNK Grouping</i>	<i>Means of $\sqrt[3]{(x+0.5)}$ transformed responses</i>	<i>n</i>	<i>Beetles</i>
A	4.18	40	20
B	3.30	40	0
C	2.08	40	120

Table 3-7. 1999 Olfactometer tests. Means separation of the $\sqrt[3]{(x+0.5)}$ transformed responses of male beetles to groups of 0, 20 and 120 male beetles on plants by Student-Newman-Keul's test. There are no significant differences between like-lettered groups. Moderate densities of males were very attractive to other males but high densities were repellent.

<i>SNK Grouping</i>	<i>Means of $\sqrt[3]{(x+0.5)}$ transformed responses</i>	<i>n</i>	<i>Beetles</i>
A	3.69	40	20
A	3.63	40	120
B	2.83	40	0

Table 3-8. 1999 Olfactometer tests. Means separation of the $\sqrt[3]{(x+0.5)}$ transformed responses of female beetles to the frass of groups of 0, 20 and 120 male beetles by Student-Newman-Keul's test. There are no significant differences between like-lettered groups. The frass of both moderate and high densities of males were very attractive to females.

<i>SNK Grouping</i>	<i>Means of $\sqrt[3]{(x+0.5)}$ transformed responses</i>	<i>n</i>	<i>Beetles</i>
A	3.91	40	120
B	2.96	40	20
B	2.86	40	0

Table 3-9. 1999 Olfactometer tests. Means separation of the $\sqrt[3]{(x+0.5)}$ transformed responses of male beetles to the frass of groups of 0, 20 and 120 male beetles by Student-Newman-Keul's test. There are no significant differences between like-lettered groups. The frass of high densities of males was very attractive to males.

<i>SNK Grouping</i>	<i>Means of $\sqrt[3]{(x+0.5)}$ transformed responses</i>	<i>n</i>	<i>Beetles</i>
A	3.33	40	120
A	2.92	40	20
A	2.86	40	0

Table 3-10. 1999 Olfactometer tests. Means separation of the $\sqrt[3]{(x+0.5)}$ transformed responses of male beetles to the frass of groups of 0, 20 and 120 female beetles by Student-Newman-Keul's test. There are no significant differences between like-lettered groups. The frass of female beetles did not influence the behaviour of male beetles at the densities tested.

Sex	Producing cues	Male		Male		Female		Female		
	Reacting to cues	Male		Female		Male		Female		
	n	32		42		38		40		
		% Success	p	% Success	p	% Success	p	% Success	p	
Monitoring time interval	15 min	1	56.3	0.11	39.5	0.06	54.8	0.1	47.5	0.12
		2	50	0.14	39.5	0.06	50	0.12	42.5	0.08
		3	53.1	0.13	42.1	0.08	50	0.12	45	0.1
		4	56.3	0.11	44.7	0.1	40.5	0.06	42.5	0.08
		5	53.1	0.13	39.5	0.06	59.5	0.06	42.5	0.08
		6	46.9	0.13	39.5	0.06	59.5	0.06	45	0.1
		7	50	0.14	31.6	0.01	50	0.12	47.5	0.12
		8	50	0.14	39.5	0.06	59.5	0.06	35	0.02
		9	46.9	0.13	39.5	0.06	47.6	0.12	45	0.1
		10	62.5	0.05	39.5	0.06	52.4	0.12	47.5	0.12
		11	62.5	0.05	47.4	0.12	40.5	0.06	47.5	0.12
	180 min	12	56.3	0.11	44.7	0.1	47.6	0.12	42.5	0.08

Table 3-11. Vial tests, May 1999. Responses of *A. nigriscutis* to male and female-associated conspecific cues. Male and female beetles were allowed to choose between untreated pieces of filter paper and those that had been exposed to male or female conspecifics for one day. A beetle was placed in a vessel consisting of a vial containing a piece of filter paper that had been exposed to a male or female conspecific that was attached to another vial containing a piece of virgin filter paper. Beetles were allowed thirty minutes to acclimate and then their positions, on treated or untreated sides, were recorded as binomial data every fifteen minutes for 3 hr. The proportion of successes, beetles on the treated side, was fit to a normal approximation of the binomial distribution. With the exception of and apparently repellent effect of males on females at one monitoring interval, none of the beetles responded to conspecific cues.

Sex	Producing cues	Male		Male		Female		Female	
	Reacting to cues	Male		Female		Male		Female	
	<i>n</i>	30		30		30		30	
		% Success	<i>p</i>	% Success	<i>p</i>	% Success	<i>p</i>	% Success	<i>p</i>
Monitoring time interval	15 min								
	1	50	0.14	73.3	0.01	36.7	0.05	60	0.08
	2	50	0.14	80	0	36.7	0.05	53.3	0.14
	3	43.3	0.11	66.7	0.03	36.7	0.05	63.3	0.05
	4	43.3	0.11	66.7	0.03	36.7	0.05	60	0.08
	5	40	0.08	80	0	36.7	0.05	60	0.08
	6	43.3	0.11	70	0.01	40	0.08	63.3	0.05
	7	43.3	0.11	73.3	0.01	50	0.14	60	0.08
	8	40	0.08	70	0.01	46.7	0.14	70	0.01
	9	43.3	0.11	66.7	0.03	50	0.14	56.7	0.11
	10	46.7	0.14	66.7	0.03	53.3	0.14	60	0.08
180 min	11	46.7	0.14	70	0.01	53.3	0.14	53.3	0.14
	12	50	0.14	70	0.01	43.3	0.11	60	0.08

Table 3-12. Vial tests, August 1999. Responses of *A. nigriscutis* to male and female-associated conspecific cues. Male and female beetles were allowed to choose between untreated pieces of filter paper and those that had been exposed to male or female conspecifics for one day. A beetle was placed in a vessel consisting of a vial containing a piece of filter paper that had been exposed to a male or female conspecific that was attached to another vial containing a piece of virgin filter paper. Beetles were allowed thirty minutes to acclimate and then their positions, on treated or untreated sides, were recorded as binomial data every fifteen minutes for 3 hr. The proportion of successes, beetles on the treated side, was fit to a normal approximation of the binomial distribution. With the exception of one time interval that females responded to female-associated cues, only females responded to cues produced by males.

<i>SNK Grouping</i>	<i>Mean Beetles Captured</i>	<i>n</i>	<i>Bait</i>
A	28.5	4	20 conspecifics
B	16.8	4	Frass of 20 conspecifics
BC	15.3	4	Mechanically damaged plant
C	9.0	4	Undamaged plant
C	8.3	4	Pot of soil

Table 3-13. 1999 Field Trapping Experiment, test 1. Means separation of the total numbers of beetles captured in traps laden with various baits by Student-Newman-Keul's test. There are no significant differences between like-lettered groups.

<i>SNK Grouping</i>	<i>Mean Beetles Captured</i>	<i>n</i>	<i>Bait</i>
A	10.0	4	20 conspecifics
AB	7.5	4	Frass of 20 conspecifics
B	6.5	4	Mechanically damaged plant
C	2.8	4	Undamaged plant
C	1.8	4	Pot of soil

Table 3-14. 1999 Field Trapping Experiment, test 1. Means separation of the numbers of male beetles captured in traps laden with various baits by Student-Newman-Keul's test. There are no significant differences between like-lettered groups.

<i>SNK Grouping</i>	<i>Mean Beetles Captured</i>	<i>n</i>	<i>Bait</i>
A	18.5	4	20 conspecifics
B	9.3	4	Frass of 20 conspecifics
B	8.8	4	Mechanically damaged plant
B	7.3	4	Undamaged plant
B	5.5	4	Pot of soil

Table 3-15. 1999 Field Trapping Experiment, test 1. Means separation of the numbers of female beetles captured in traps laden with various baits by Student-Newman-Keul's test. There are no significant differences between like-lettered groups.

<i>SNK Grouping</i>	<i>Mean Beetles Captured</i>	<i>n</i>	<i>Bait</i>
A	12.8	4	20 conspecifics
A	9.0	4	Pot of soil
A	7.8	4	Plant
A	6.0	4	Frass of 20 <i>Aphthona lacertosa</i>
A	5.3	4	20 <i>Aphthona lacertosa</i>

Table 3-16. 1999 Field Trapping Experiment, test 2. Means separation of the total numbers of beetles captured in traps laden with various baits by Student-Newman-Keul's test. There are no significant differences between like-lettered groups. Differences between treatments approached significance, $p = 0.0604$. Results may have been offset by very cool, windy conditions.

<i>SNK Grouping</i>	<i>Mean Beetles Captured</i>	<i>n</i>	<i>Bait</i>
A	7.8	4	20 conspecifics
A	7.5	4	Pot of soil
A	5.3	4	Plant
A	3.8	4	Frass of 20 <i>Aphthona lacertosa</i>
A	3.3	4	20 <i>Aphthona lacertosa</i>

Table 3-17. 1999 Field Trapping Experiment, test 2. Means separation of the numbers of female beetles captured in traps laden with various baits by Student-Newman-Keul's test. There are no significant differences between like-lettered groups.

<i>SNK Grouping</i>	<i>Mean Beetles Captured</i>	<i>n</i>	<i>Bait</i>
A	5.0	4	20 conspecifics
B	2.5	4	Plant
B	2.3	4	Frass of 20 <i>Aphthona lacertosa</i>
B	2.0	4	20 <i>Aphthona lacertosa</i>
B	1.5	4	Pot of soil

Table 3-18. 1999 Field Trapping Experiment, test 2. Means separation of the numbers of male beetles captured in traps laden with various baits by Student-Newman-Keul's test. There are no significant differences between like-lettered groups.

<i>SNK Grouping</i>	<i>Mean Beetles Captured</i>	<i>n</i>	<i>Bait</i>
A	14.3	4	20 conspecific males
B	8.3	4	Undamaged plant
B	7.8	4	20 conspecific females
B	5.5	4	Pot of soil

Table 3-19. 1999 Field Trapping Experiment, test 3. Means separation of the total numbers of beetles captured in traps laden with various baits by Student-Newman-Keul's test. There are no significant differences between like-lettered groups.

<i>SNK Grouping</i>	<i>Mean Beetles Captured</i>	<i>n</i>	<i>Bait</i>
A	8.3	4	20 conspecific males
AB	5.0	4	Undamaged plant
AB	4.0	4	20 conspecific females
B	2.8	4	Pot of soil

Table 3-20. 1999 Field Trapping Experiment, test 3. Means separation of the numbers of female beetles captured in traps laden with various baits by Student-Newman-Keul's test. There are no significant differences between like-lettered groups.

<i>SNK Grouping</i>	<i>Mean Beetles Captured</i>	<i>n</i>	<i>Bait</i>
A	6.0	4	20 conspecific males
A	3.8	4	20 conspecific females
A	3.3	4	Undamaged plant
A	2.8	4	Pot of soil

Table 3-21. 1999 Field Trapping Experiment, test 3. Means separation of the numbers of male beetles captured in traps laden with various baits by Student-Newman-Keul's test. There are no significant differences between like-lettered groups.

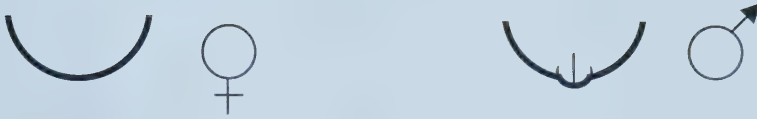
Figures

Figure 3-1. Diagram representing a ventral view of the last abdominal sternite of *A. nigriscutis*. Beetles were sexed by examining this sternite. Those of male beetles have a distinctly lobed appearance and a medial line.

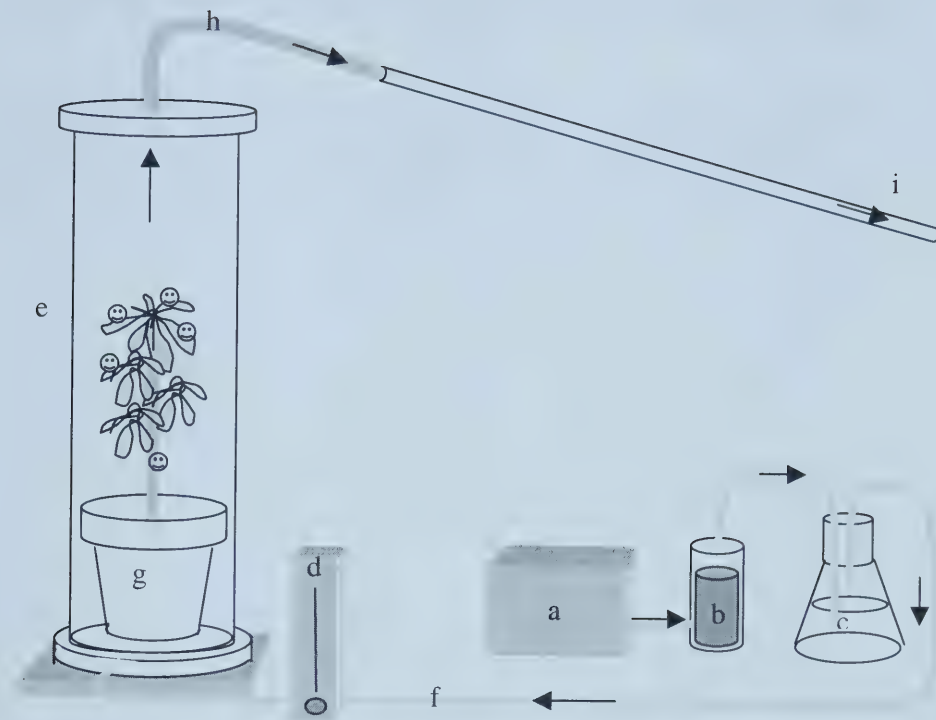


Figure 3-2. Olfactometer. Air from a pump (a) was forced through activated charcoal (b), distilled water (c) and a flow regulator (d) to the 1m by 25 cm chamber (e). These devices were connected by clear nylon tubing (2.5 mm internal diameter) (f). Air flowed through the chamber, past test materials (g -in this case plants with beetles) to a 1 cm diameter hole in its top. Flexible tubing (h) connected the chamber to a 1m-glass tube (1 cm internal diameter). Air flowed to its open end (i). Individual beetles were introduced to this end. The glass tube was marked at 10 cm intervals. Arrows represent direction of airflow. Happy faces represent beetles.

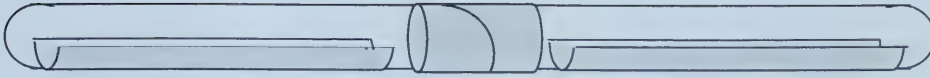


Figure 3-3. Two 1.5 by 6-cm vials, attached at their open ends with tape and each containing a piece of filter paper. The paper in one vial was exposed to a conspecific for 24 hr. The other was untreated. A beetle on the treated side was recorded as a success.

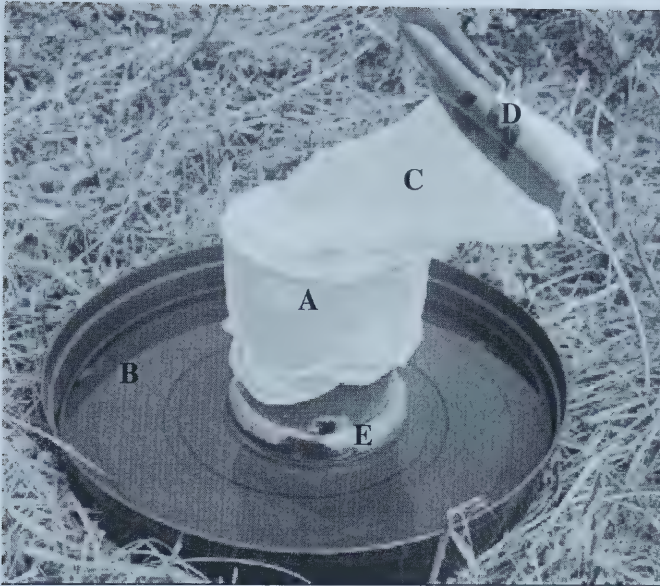


Figure 3-4. Soapy water trap. The attractiveness of test materials was assessed in the field with these traps. Insects were drawn to the test materials and trapped in the soapy water. A-Test material inside a 6in plastic pot. B-60 cm diameter by 12 cm deep plastic tray. The tray was filled with soapy water and captured insects that were attracted to the traps. C- Mesh bag secured to the pot with an elastic band. This bag allowed volatiles to escape and prevented the escape of test insects and access of field insects to the test materials. D- Plastic pin-flag to keep mesh bag upright. E- plastic tray to keep the pot out of the water.



Figure 3-5. Soapy water traps arranged in a complete randomised block array near the Beverly Bridge release sites in Edmonton, Alberta. Traps were set approximately 2 m from a thick strip of leafy spurge (to the right of the traps in this photo), inside a mowed strip at the bottom of a hill and 5 m apart. Up to five treatments were tested at a time and each was replicated four times. Traps were monitored after 48 hr of being set.

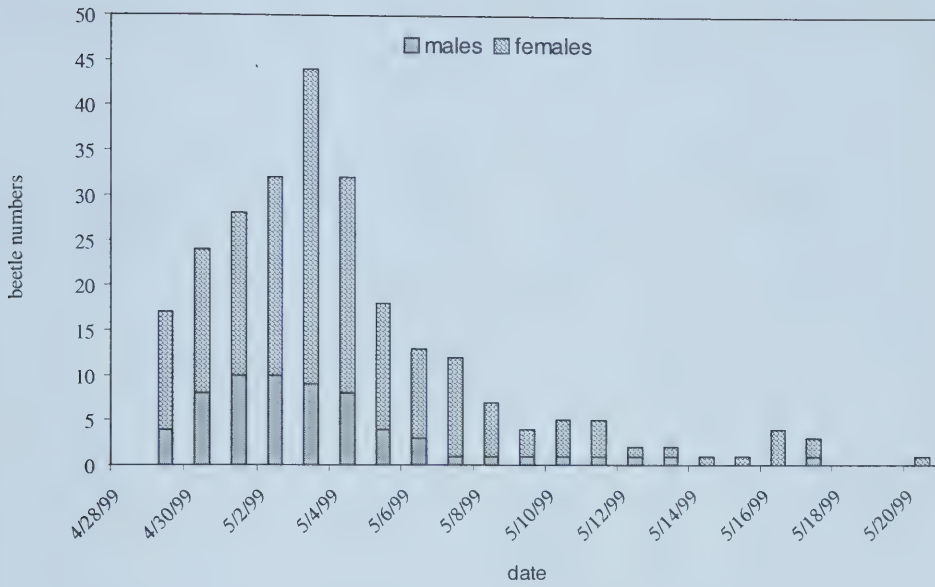


Figure 3-6. Emergence of adult *A. nigriscutis* from soil cores collected from Edmonton in April 1999. 255 beetles emerged. 74.9% were female.

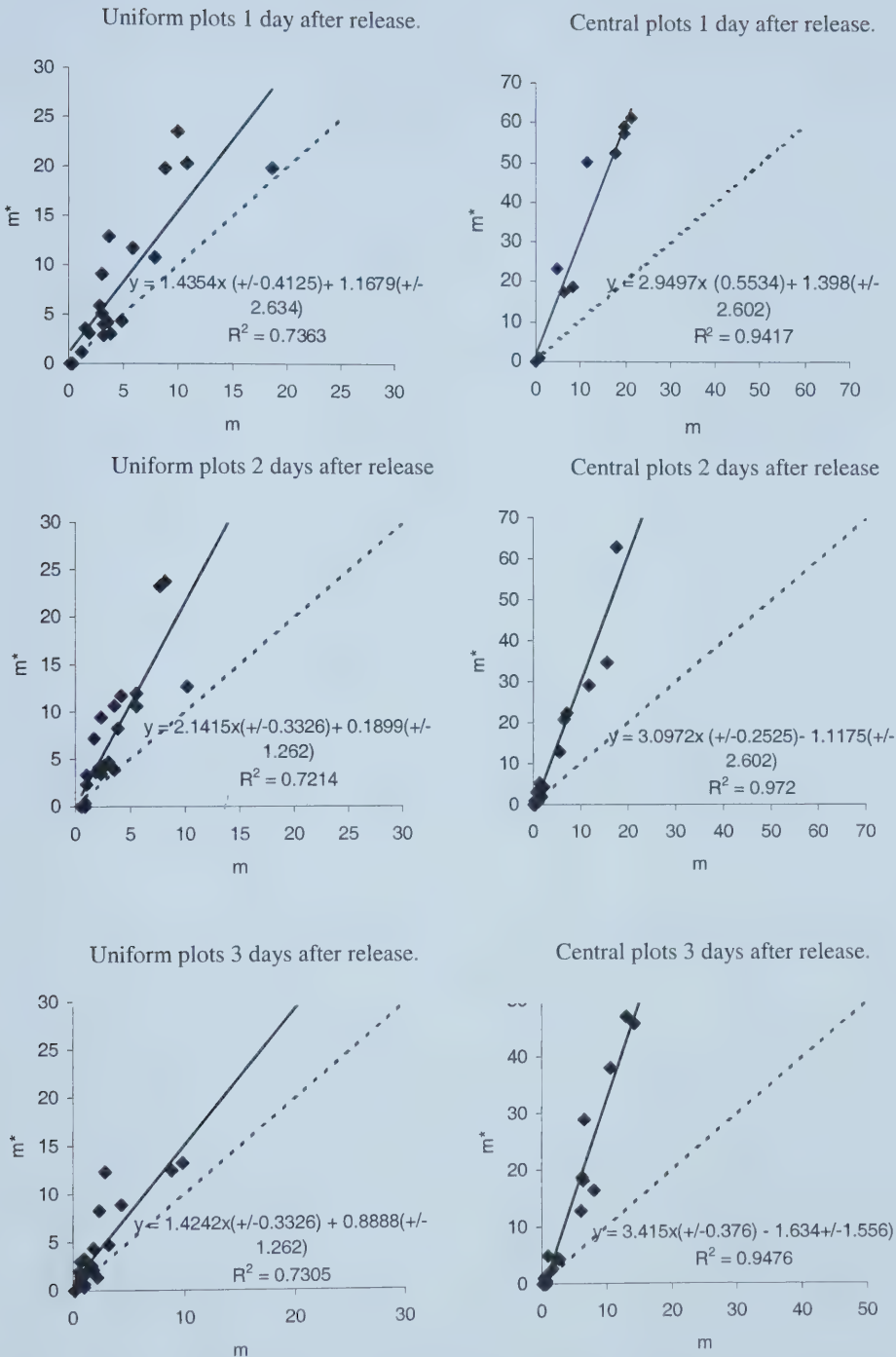


Figure 3-7. Iwao's regression of Lloyd's mean crowding coefficient by the mean numbers of beetles column of each plot for central and uniform releases and associated 95% confidence intervals 1, 2 and 3 days after release. There were significant differences in the levels of aggregation of beetles on central and uniform plots at these times. Beetles were more aggregated on central plots.

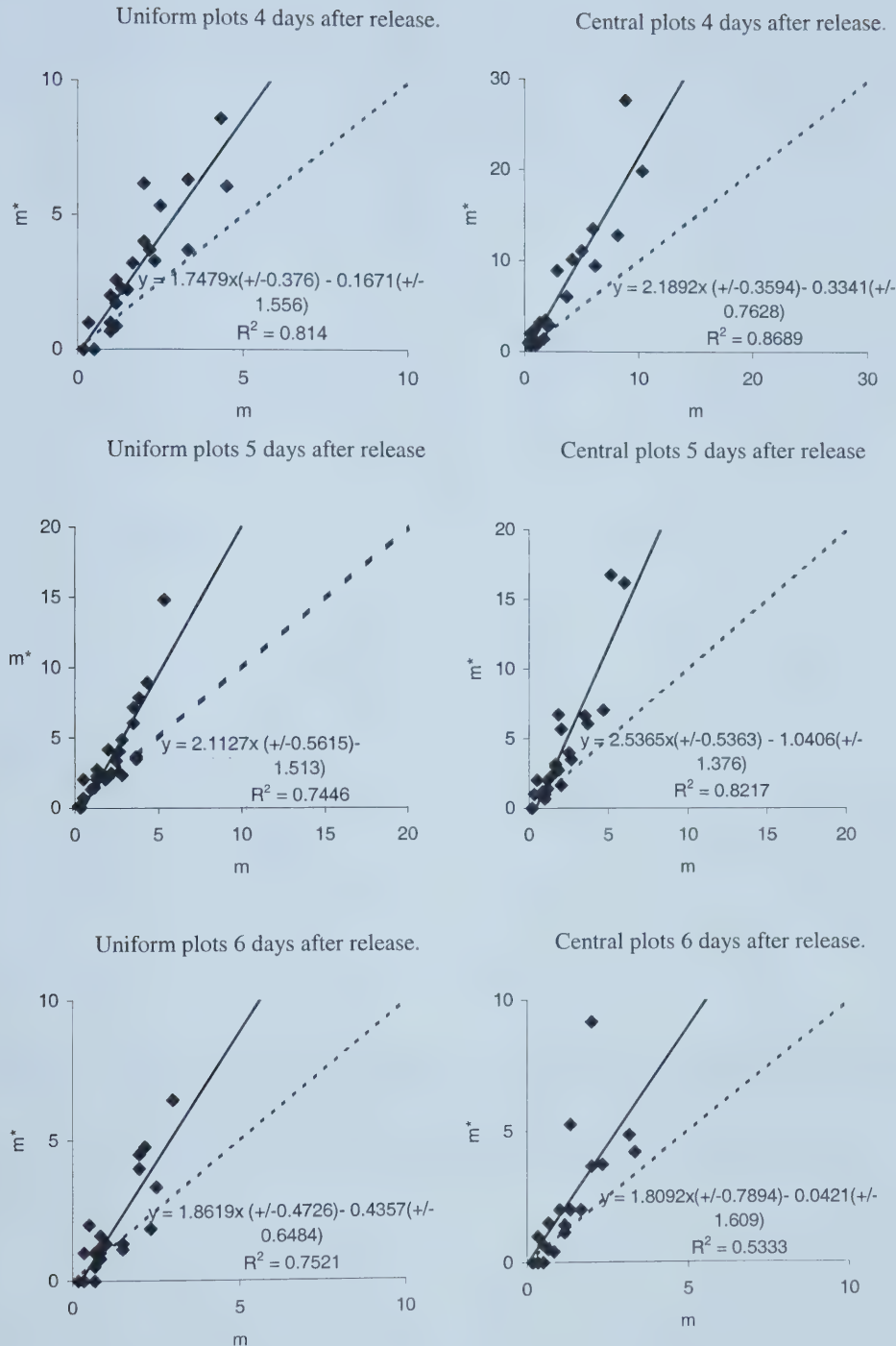
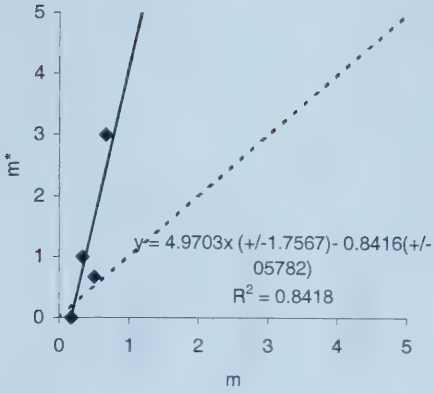
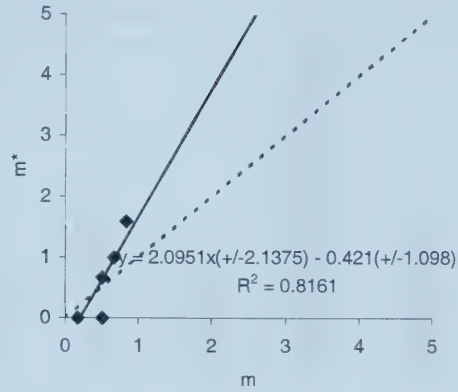


Figure 3-8. Iwao's regression of Lloyd's mean crowding coefficient by the mean numbers of beetles column of each plot for central and uniform releases and associated 95% confidence intervals 4, 5 and 6 days after release. There were no significant differences in the levels of aggregation of beetles on these plots at these times.

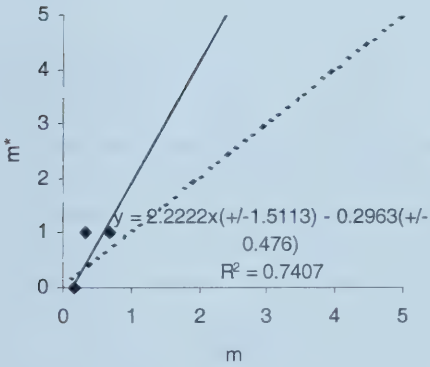
Uniform plots 21 days after release.



Central plots 21 days after release.



Uniform plots 39 days after release.



Central plots 39 days after release.

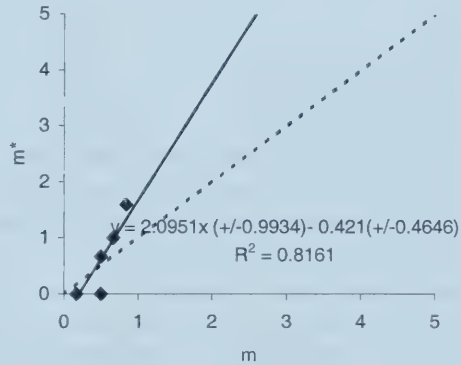


Figure 3-9. Iwao's regression of Lloyd's mean crowding coefficient by the mean numbers of beetles column of each plot for central and uniform releases and associated 95% confidence intervals 21 and 39 days after release. There were no significant differences in the levels of aggregation of beetles on these plots at these times.

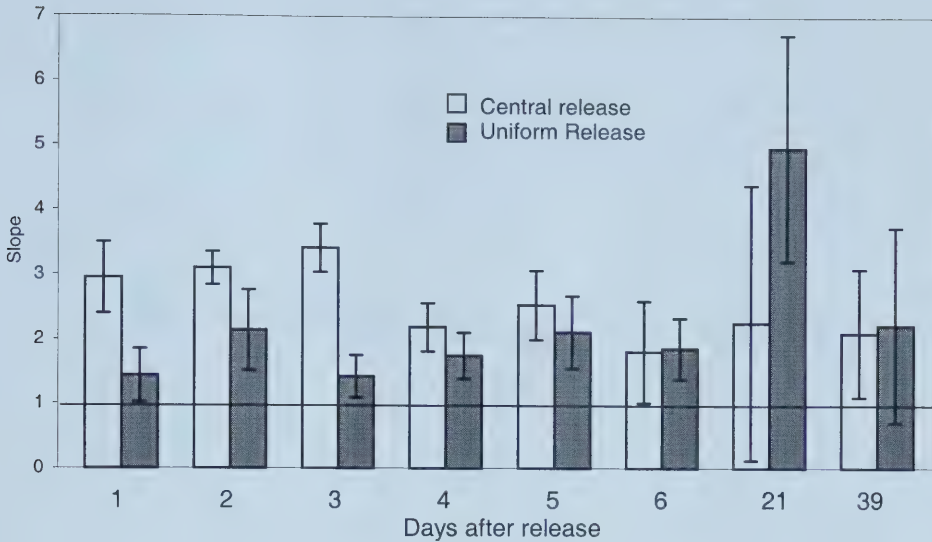


Figure 3-10. Summary of the slopes and associated 95% confidence intervals of functions associated with the regression of Lloyd's mean crowding coefficient with the mean numbers of beetles per column of each plot. The slopes of these functions indicate the level of aggregation on plots at each time. Values significantly above 1 indicate aggregation, those not significantly different than 1 are Poisson distributed and those significantly lower than 1 are uniformly distributed. Beetles were aggregated, though not to the same extent on central and uniform release plots from one to three days after release. Beetles were also aggregated, without significant differences in the levels of aggregation, from day 4 to day 6. Although there were no significant differences in the levels of aggregation on plots at 21 and 39 days after release, there were also no differences in distribution from random on central plots 21 days and uniform releases 39 days after release.

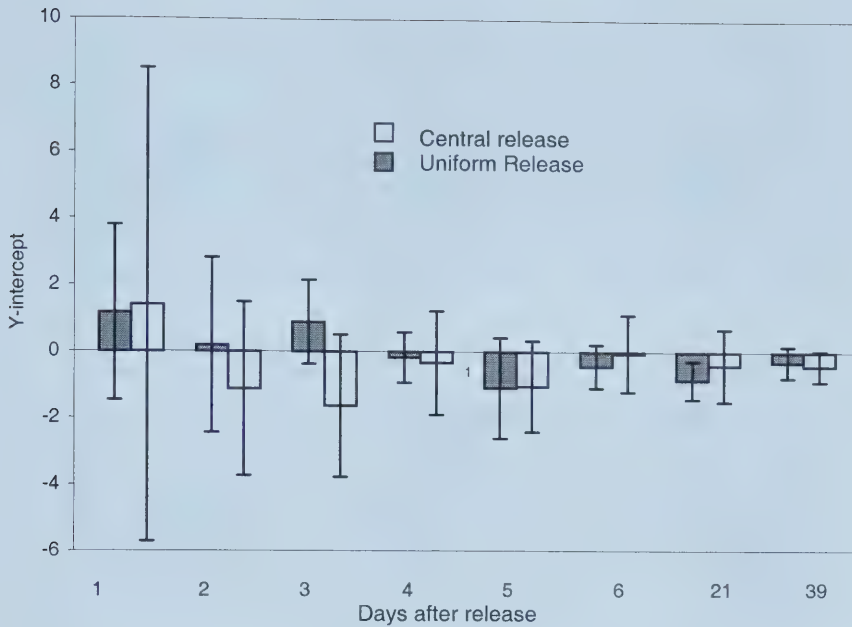


Figure 3-11. Summary of the Y-intercepts and associated 95% confidence intervals of functions associated with the regression of Lloyd's mean crowding coefficient with the mean numbers of beetles per column of each plot. The values of the Y-intercepts indicate the numbers of beetles per congregation. Values greater than 0 indicate congregations of more than one beetle, values of 0 indicate that the individual is the 'basic component of the distribution' is the individual and value less than 0 indicate repulsion. Results were inconclusive as, with the exception of that associated with beetles released on a central point on plots 21 days after release, none of the Y-intercept values were significantly different from 0 and most were not significantly different from 1.

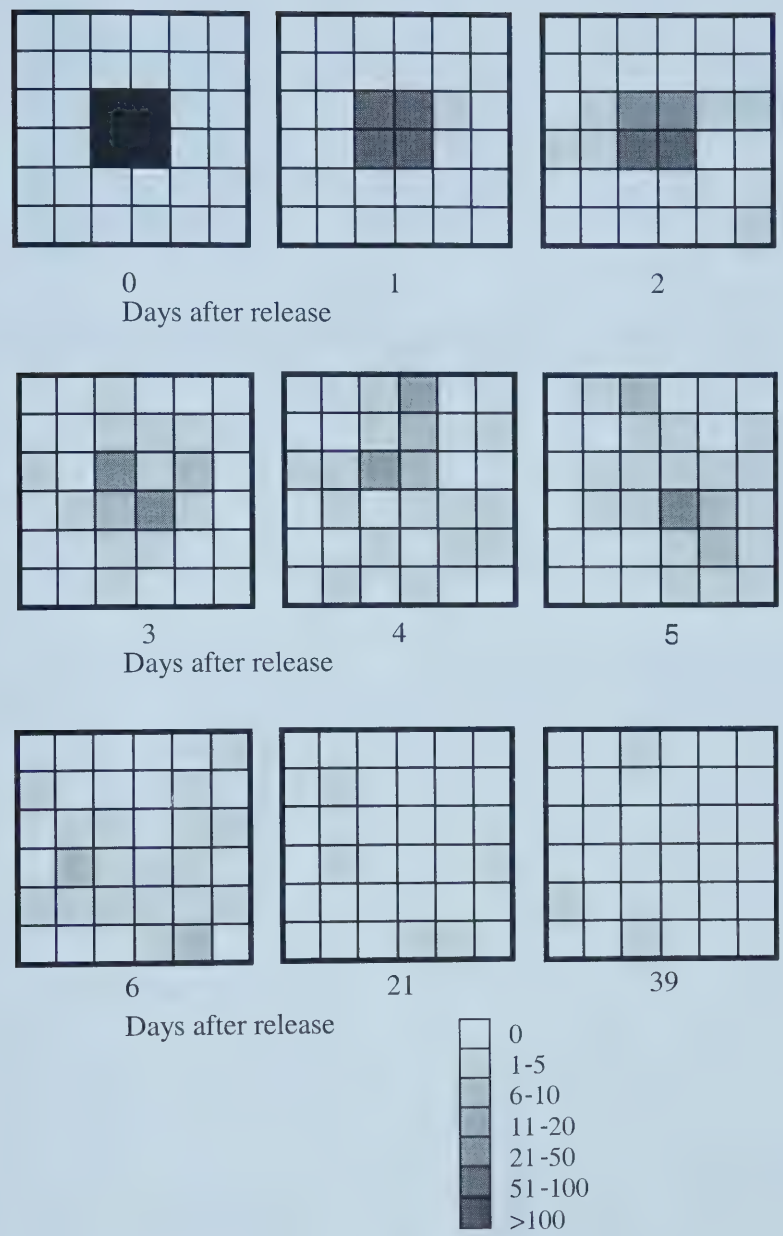


Figure 3-12. Beetle distribution over a 39-day period after a central release of 1000 beetles on a 2m by 2m plot in a flat, leafy spurge infested field. Each plot was subdivided into 36 equal, 33 cm X 33 cm subplots.

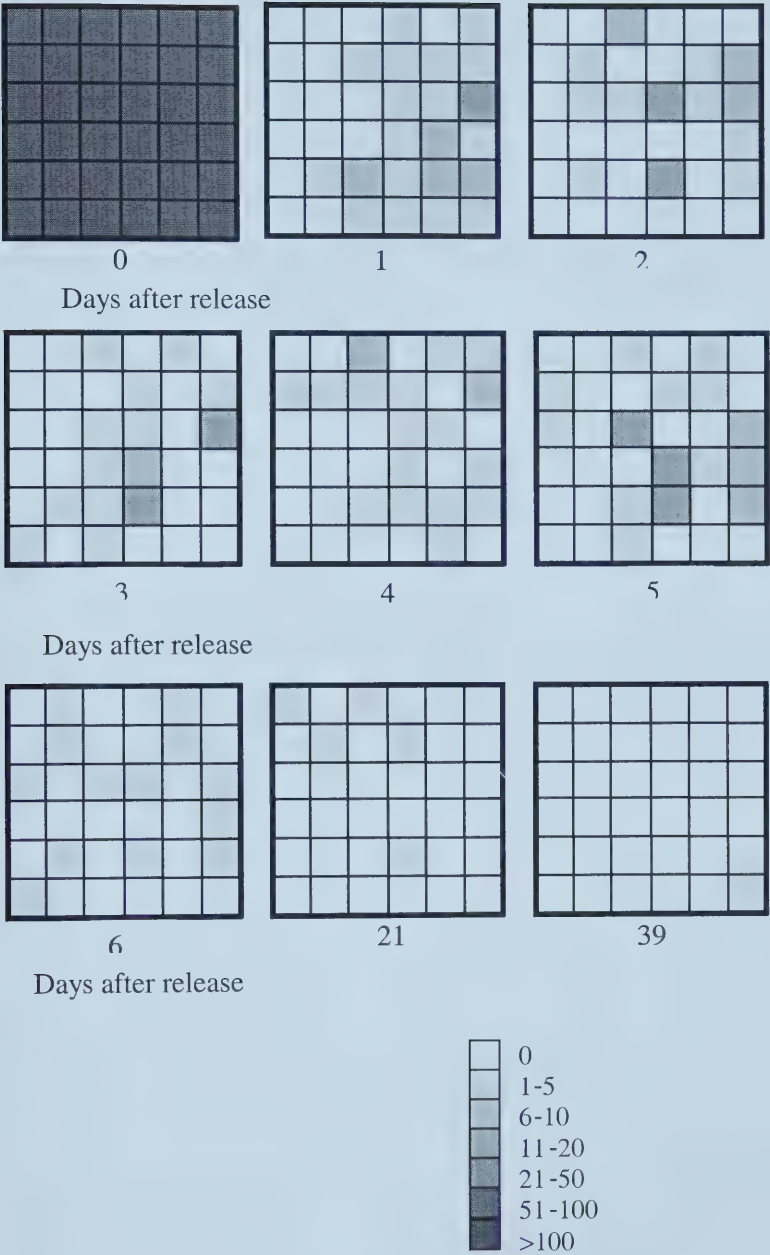


Figure 3-13. Beetle distribution over a 39-day period after a uniform release of 1000 beetles on a 2m by 2m plot in a flat, leafy spurge infested field. Each plot was subdivided into 36 equal, 33 cm X 33 cm subplots.

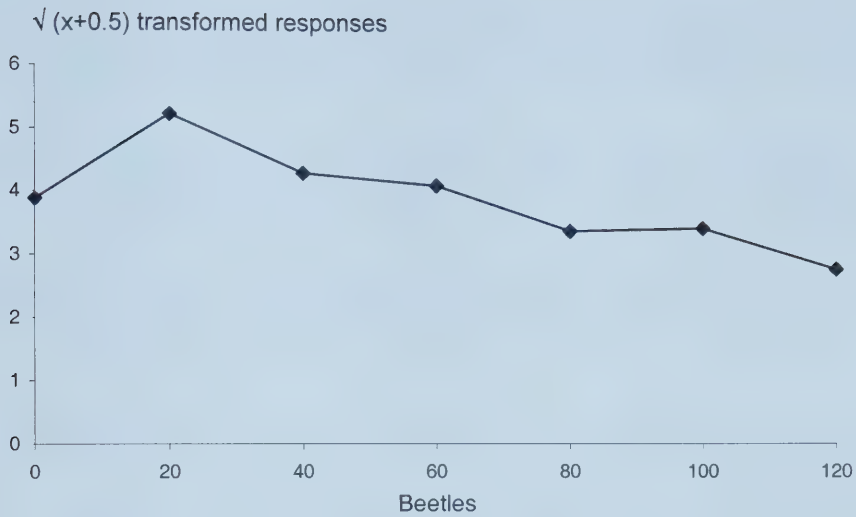


Figure 3-14. The $\sqrt{x+0.5}$ transformed responses of beetles to increasing numbers of conspecifics on plants. Beetles at relatively low densities were highly attractive to conspecifics. This attraction fell off with increasing densities to repulsion at high densities.

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Chapter 4

Leafy Spurge Variability: Effects on *Aphthona nigriscutis* Congregation and Dispersal Cues.

Introduction

A significant proportion of *Aphthona nigriscutis* releases on leafy spurge in North America have not established (McClay et al. 1995, Hansen et al. 1997). Adult *A. nigriscutis* are relatively host-specific (Gassman 1996) and discriminate among leafy spurge from different Albertan collection sites (Chapter 2). Within-site variability of leafy spurge may be a factor that contributes to establishment by promoting congregation behaviour in these beetles. As congregation behaviour has been implicated as an important factor in establishment of some biological control agent populations (Beddington et al. 1978, cited from Begon et al. 1990), effects of plant factors on this behaviour may influence *A. nigriscutis* establishment. *A. nigriscutis* congregation behaviour is influenced by an aggregation pheromone. The attractiveness of this pheromone is influenced by substrate (Chapter 3) and may also be subject to similar variable host plant factors as feeding preferences.

There are relevant differences between the responses of *A. nigriscutis* to some North American leafy spurge samples and European spurge species that are the sources of these biological control agents. Collections of *A. nigriscutis* in Europe indicated a close association of *A. nigriscutis* adults with *Euphorbia cyparissias* and *E. seguieriana* (Maw 1981, Gassman et al. 1996). The hybrid species complex considered predominant in Canada by Harris et al. (1985) is *E. x pseudovirgata*. Larval survival tests indicated differences in the suitability of several spurge species, including a collection of North American leafy spurge, as hosts for *A. nigriscutis*. First instar beetle larvae were transferred to North

American leafy spurge, *E. cyparissias* and *E. seguieriana*. Roughly three times more larvae survived until the third instar upon *E. esula* and *E. seguieriana* than on the North American leafy spurge tested (Gassman et al. 1996). Although *A. nigriscutis* larvae did not completely reject the North American leafy spurge tested, differences in larval survival may indicate antibiosis resistance.

There are also relevant differences among North American leafy spurge samples. In Chapter 2, effects of host plant variability were indirectly examined with a series of feeding choice tests. These tests demonstrated that there were significant within- and among-site differences in feeding preferences of *A. nigriscutis* for leafy spurge samples collected from several previous beetle release sites in Alberta (Chapter 2). Causes of variable palatability have yet to be determined. However, because feeding preferences were demonstrated when propagation conditions and growth medium were shared by all leafy spurge samples, they were likely stable and genetically determined. The maintenance of these differences over two years supports this conclusion. Rowe et al. (1997) demonstrated genetic differences among and within North American leafy spurge populations that were profound enough to suggest multiple introductions.

Variable surface or near-surface plant chemistry demonstrated by Manners and Davis (1984) and variable latex triterpenoid profiles of leafy spurge demonstrated by Holden and Mahlberg (1992) have the potential to influence feeding preferences and, as these characteristics were used for chemotaxonomic evaluation, are likely genetically determined. Manners and Davis (1984) demonstrated variations in the leaf wax concentrations of the potentially

antifeedant triterpenoids α -amyrin and β -amyrin, both among North American samples as well as between North American and Austrian samples.

The triterpenoids α -amyrin and β -amyrin were found to be present in higher concentrations in azalea varieties resistant to the azalea lace bug *Stephanitis pyrioides* (Heteroptera: Tingidae) (Balsdon et al. 1995) and crucifers resistant to diamondback moth larvae, *Plutella xylostella* (Lepidoptera: Plutellidae) (Eigenbrode et al. 1991) than in susceptible varieties. Concentrations of these compounds may also affect host selection by *A. nigriscutis* and impart anti-xenosis resistance to these insects upon some leafy spurges.

Painter (1951) described non-preference, later called anti-xenosis by Kogan and Ortman (1978, cited from Palaniswamy et al. 1992), as a group of plant characteristics that discourage the use of a plant as food, oviposition substrate or shelter by an herbivorous insect. Although all of the leafy spurge tested in Chapter 2 suffered feeding damage and oviposition and are likely acceptable hosts for *A. nigriscutis*, variable preferences for these plants may have implications for *A. nigriscutis* congregation and dispersal behaviours.

When insect aggregations occur in response to cues produced by conspecifics, Turchin (1998) describes them as congregations. Congregations result from behavioural responses of organisms to audible, visual or chemical cues that act independently or in combination. Many examples of chemically-mediated congregation have been documented in Coleoptera and include beetles in the families Scolytidae (Renwick and Vité 1969), Bostrichidae (Fadamiro et al.,

1996), Curculionidae (Perez et al.. 1997), Scarabaeidae (Yarden and Shani 1994), and Nitidulidae (Petroski and Vaz 1995).

Within Chrysomelidae, members of the subfamily Alticinae, *Phyllotreta cruciferae* (Peng and Weiss 1992) and *Longitarsus jacobaeae* (Zhang and McEvoy 1996) are attracted to conspecific chemical cues, aggregation pheromones, and form congregations. Both *A. nigriscutis* congregation and dispersal behaviours are influenced by pheromones (Chapter 3). *A. nigriscutis* are highly attracted to conspecifics at relatively low densities and repelled by higher densities.

Substrate, the presence or absence of leafy spurge plants, influences the attractiveness of *A. nigriscutis* to conspecifics (Chapter 3). Relatively low densities of beetles on plants were more attractive than those without plants. However, mechanically damaged plants from the sample tested were no more attractive to *A. nigriscutis* than undamaged plants. Differences in the responses of these beetles to same-size groups with or without these plants are not due to the attractive effects of plant volatiles. Differences in responses of *A. nigriscutis* to conspecifics are likely due to differences in pheromone production in response to or derived from a plant substrate. Peng and Weiss (1992) demonstrated that *P. cruciferae* requires contact with or feeding upon a suitable host plant for production of its aggregation pheromone.

Differences in substrate, based on host plant chemistry, may also influence the attractiveness of an aggregation pheromone. Females of the European elm bark beetle, *Scolytus multistriatus* (Scolytidae) produce an aggregation

pheromone. This pheromone consists of 4-methyl-3-hepanol and (-)- α -multistriatin, both produced by *S. multistriatus* and (-)- α -cubebene, produced by the host tree (Gore et al. 1977, cited from Lanier 1983 in Ahmad 1983). Lanier (1983, in Ahmad 1983) suggests that potential host trees that do not produce (-)- α -cubebene when attacked do not contribute to the aggregation pheromone and are not extensively colonized.

There may also be differences in host plant-produced pheromone precursors. Production of components of *Ips paraconfusus* (Coleoptera: Scolytidae) aggregation pheromone requires exposure to vapours of the host tree monoterpene, myrcene (Hughes 1974). The production and attractiveness of *A. nigriscutis* aggregation pheromone may also be subject to host plant factors.

Host plant volatiles may also influence *A. nigriscutis* congregation behaviour. Host plant volatiles are attractive to *Carpophilus hemipterus* (Nitidulidae) (Petroski and Vaz 1995) and *Psylliodes chrysocephala* (Chrysomelidae: Alticinae) (Koritsas et al. 1991), the latter being attracted to mechanically damaged host plants. *Diabrotica* spp. (Chrysomelidae) are also attracted to host plant volatiles, tetracyclic triterpene cucurbitacins. These compounds act as arrestants and feeding stimulants for these cucurbit specialists. Attack and feeding are directly related to the amounts of cucurbitacins B and E (Metcalf et al. 1979). As effects of leafy spurge volatiles on *A. nigriscutis* behaviour were tested using clones of the leafy spurge sample determined to be the least preferred host identified in feeding trials, discounting the effects of

potentially variable volatile plant compounds on the congregation and dispersal behaviours of *A. nigriscutis* would be premature.

An interaction of pheromones and plant volatiles, where effects of pheromones are enhanced by host plant volatile emissions, has been described for a number of insect-host systems (for example, Bogner et al. 1992) and include beetles in at least four families, Scarabaeidae (Yarden and Shani 1994), Nitidulidae (Dowd and Bartelt 1991, Lin et al. 1992), Curculionidae (Weissling et al. 1993) and Scolytidae (Wood 1982). Although no evidence for such interactions was evident in previous tests of *A. nigriscutis* responses, these test plants were again the least palatable of the feeding trials and did not produce attractive volatiles. Interaction of plant volatiles and *A. nigriscutis* pheromones and the resulting responses of nearby conspecifics may vary as host surface (Manners and Davis 1984) and latex (Holden and Mahlberg 1992) compounds and feeding preferences do.

Variable production of conspecific cues, variable plant volatiles and interactions among these factors may have consequences for *A. nigriscutis* congregation behaviour. *A. nigriscutis* were shown to form relatively short-lived congregations when released on field plots (Chapter 3). Numbers in these groups increased, decreased and relocated about test plots on a daily basis. These cycles of metapopulation growth and dispersal are likely influenced by the responses of *A. nigriscutis* to attractive and repulsive cues produced by conspecifics (Chapter 3). Production or reception of aggregation pheromone, or interaction of this compound(s) and plant volatiles, could prove to be as variable as the feeding

preferences of *A. nigriscutis*. Should this be the case, differences in plant chemistry, as implied by the results of feeding trials, could result in differences in congregation and dispersal behaviours of *A. nigriscutis* on variable host plants.

Laboratory olfactometer bioassays were used to assess potential differences in the attractiveness of volatiles associated with leafy spurge plants from several collection sites to *A. nigriscutis*. Effects of leafy spurge biotype on the attractiveness of *A. nigriscutis* pheromones were also examined. A field test was used to assess the effects of variably palatable plants on adult beetle distribution.

Methods and Materials

Beetles. Adult *A. nigriscutis* were collected with sweep nets from a site near the Beverly Bridge in Edmonton, Alberta from June to September 1999. *A. nigriscutis* were maintained on potted Cardston (ARC) leafy spurge plants in mesh cages in the laboratory for a minimum of 24 hr prior to their participation in experiments and were starved for 24 hr before use. Individual beetles participated in olfactometer tests only once.

Plants. *Plant Collection and Propagation, 1998.* Plants were collected from nine large leafy spurge infestations in eight municipalities in southern and central Alberta during May 1998. Collections were made in or near Edmonton, Ponoka, Hardisty, Olds, Fort Macleod, Lethbridge, Millet, and two sites near Cluney, Alberta. *A. nigriscutis* had been released on each of these sites at least seven years before this study and regularly monitored for at least three years following release. Beetle establishment, based on the maximum numbers recovered in subsequent years, varied from site to site (unpublished data, courtesy Alec McClay). These recoveries were used to categorize each site into one of three establishment classes - *good* with 100 or more beetles in 20 sweeps, *moderate* with 6 to 99, and *poor* with 0 to 5 beetles.

Plants were collected from three sites from each establishment category. Leafy spurge plants were also obtained from Dr. Alec McClay of the Alberta Research Council, Vegreville. Hereafter, these plants are referred to as Cardston (ARC). These plants were originally collected from a release site with a well

established population of *A. nigriscutis* near Cardston, AB in 1991 and had since been used to rear and maintain leafy spurge biological control agents.

Individual plants were excavated. Plant top-growth was removed and the intact, approximately 2700 cm³ clumps of soil and root material were transported to a greenhouse where roots and soil were separated. To minimize variation, all plants tested from each site were grown from cuttings of a single large root from each sample. A single plant provided all of the root material for propagation of Cardston (ARC) plants. Plants propagated from each sample were clones. For each source, leafy spurge root cuttings, approximately 2 cm long by no less than 5 mm diameter, were planted in a moistened commercial growth medium (Sunshine Professional) in a 6 in diameter pot. Each large root was divided into at least ten portions to ensure adequate material for testing. These plants were collected and propagated in 1998, wintered in a greenhouse and tested in 1999.

Laboratory Olfactometer Bioassays. The same olfactometer design and bioassay detailed in Chapter 3 was used to assess the responses of *A. nigriscutis* to each test material.

Plant Volatiles, Undamaged Plants. *A. nigriscutis* fed preferentially on some of these samples in feeding trials (Chapter 2). Volatiles from undamaged clones from all ten samples collected in 1998 were assessed.

Plant Volatiles, Mechanical Damage. The attractiveness of volatiles associated with mechanically damaged plants was assessed. Small pieces were removed with scissors from 2, 4, 8 and 16 leaves to simulate increasing levels of feeding damage. Responses of *A. nigriscutis* to these plants were compared to those to undamaged plants. The plant samples Ponoka, Fort Macleod and Cardston (ARC) were tested. Previous feeding trials results indicated that the Ponoka sample was the most palatable, Cardston (ARC) was the least and Fort Macleod was intermediate. Statistically significant differences in levels of feeding damage were observed between Ponoka and Cardston (ARC) and Fort Macleod and Cardston (ARC) plants.

Plant Volatiles and A. nigriscutis Pheromones. Effects of *A. nigriscutis* pheromones on conspecific behaviours as well as those of substrate on the production of these cues have been observed (Chapter 3). Tests here were conducted to assess potential differences in responses of *A. nigriscutis* to conspecifics feeding upon leafy spurge plants that have significant differences in palatability.

Responses of *A. nigriscutis* to Ponoka and Fort Macleod plants with groups of 20, 40, 60, 80, 100 and 120 conspecifics were assessed and compared to responses to undamaged clones from these samples. Results were compared to the responses of *A. nigriscutis* to same-size groups of conspecifics on Cardston (ARC) clones.

Field Plot Aggregation Study. *A. nigriscutis* distributions upon arrays of potted Ponoka, Fort Macleod and Cardston (ARC) clones were examined. Pots containing individual plants were spaced 16 cm apart in a 5 by 5 plant arrays. Each array was defined as a plot and replicated four times for a total of 12 plots. Plots were separated by 12 m and arranged in a randomized complete block design. Groups of 750 beetles were released on the central plant of each plot.

Immediately before release, *A. nigriscutis* were marked with a fluorescent powder (see Chapter 3). Each plot was assigned one of six colours. Adjacent plots were assigned different colours to allow identification of *A. nigriscutis* migrating from neighbouring plots. This experiment was conducted at the University of Alberta, Ellerslie research farm in a canary grass (*Phalaris canariensis* L) field. Canary grass was 40 cm high when the experiment was conducted. Potted plants were placed in 6 in diameter plastic trays to reduce the risk of inoculating the experimental site with leafy spurge. These trays did not inhibit beetle movement.

The numbers of *A. nigriscutis* per plot and numbers of immigrant *A. nigriscutis* from other plots were assessed by visual counts of beetle numbers per plant at 4, 24 and 144 hr intervals following release. Spatial distribution of *A. nigriscutis* within each plot was also assessed at these time intervals.

Data Analysis.

Laboratory Olfactometer Bioassays. Olfactometer results were transformed by $\sqrt{(x+0.5)}$ to stabilize variance. Transformed data were compared by ANOVA.

Field Plot Aggregation Study. For field experiments, total and immigrant *A. nigriscutis* numbers per plot were compared by ANOVA. *A. nigriscutis* spatial distribution on each leafy spurge biotype at each time interval was assessed and compared using Iwao's (1968) regression method (for details of this analysis see Ch 3).

Results

Laboratory Olfactometer Bioassays.

Plant volatiles-undamaged plants. There were no significant differences in the responses of beetles to undamaged clones from these ten samples ($p=0.5378$).

Plant volatiles-mechanical damage. There were no significant differences in the responses of beetles to increased mechanical damage to Ponoka ($p=0.6507$) or Cardston (ARC) plants ($p=0.1139$). However, *A. nigriscutis* responded to increasing mechanical damage to Fort Macleod ($p=0.0001$) plants and treatments of 8 and 16 damaged leaves elicited the greatest response. There were no significant differences between responses of *A. nigriscutis* to plants with either 8 or 16 damaged leaves (Table 4-1). There were no such trends evident for increasing damage to Ponoka or Cardston (ARC) plants.

Comparisons of pooled responses of *A. nigriscutis*, as determined by the distances traveled up the olfactometer towards test materials, to all levels of mechanical damage to Ponoka, Fort Macleod and Cardston (ARC) plants indicated no significant effect of sample ($p=0.1861$) although a significant sample by damage level interaction was apparent ($p=0.0001$). *A. nigriscutis* responded differently to volatiles from mechanically damaged Ponoka, Fort Macleod and Cardston (ARC) plants and responded positively to increased levels of damage on Fort Macleod plants only.

Plant volatiles and A. nigriscutis pheromones. There were significant differences in responses of *A. nigriscutis* to conspecifics on Ponoka plants ($p=0.0001$). *A. nigriscutis* were most attracted to 20 conspecifics per plant. This attraction decreased with increasing beetle density with repellence evident at 100 beetles per plant (Table 4-2). Similar responses of *A. nigriscutis* to conspecifics on Fort Macleod plants ($p=0.0001$) were observed, though the greatest attraction occurred with 60 conspecifics per plant, attraction was maintained until 80 and repellence did not occur until 120 conspecifics per plant (Table 4-3). Overall, attraction at 80 was not significantly greater than to 20 *A. nigriscutis* on Fort Macleod plants. Attraction of *A. nigriscutis* to Cardston (ARC) plants was evident only with groups of 20 conspecifics. Groups of 40 to 100 conspecifics were not attractive and groups of 120 were repellent (Table 4-4).

Comparisons of responses of *A. nigriscutis* to conspecifics on Ponoka, Fort Macleod and Cardston (ARC) plants indicated significant sample ($p=0.0001$) and conspecific numbers ($p=0.0001$) effects as well as interaction of these factors ($p=0.0001$). Means separation using Student-Newman-Keul's test indicated that, overall, *A. nigriscutis* on Ponoka and Fort Macleod plants were more attractive to conspecifics than those on Cardston (ARC) plants (Table 4-5).

Results indicate *A. nigriscutis* responded differently to conspecifics feeding upon these plants. There was a significant interaction of sample and conspecific numbers when responses of *A. nigriscutis* to conspecifics on Fort Macleod and Cardston (ARC) plants were compared ($p=0.0002$). A significant interaction of these factors was also evident when responses to Fort Macleod and

Ponoka plants were compared ($p=0.0001$) and again when those to Ponoka and Cardston (ARC) plants were compared ($p=0.0004$). Responses of *A. nigriscutis* to conspecifics upon these three plants are summarized in Figure 4-1.

A. nigriscutis demonstrated preferences for these samples similar to those demonstrated in feeding trials in Chapter 2. Ponoka plants were preferred, followed by Fort Macleod plants. Responses of *A. nigriscutis* to conspecifics upon both of these were significantly greater than those to conspecifics upon Cardston (ARC) plants (Table 4-6).

Field Plot Aggregation Study. Both the 95% confidence intervals of the slope and intercept of the regression of Lloyd's "mean crowding" (Lloyd 1967) to mean numbers of beetles per column, per sample, per monitoring time interval and Iwao's patchiness regression (Iwao 1968, cited from Christensen 1977), revealed that *A. nigriscutis* assumed aggregated distributions on all plants at all time intervals with the exception of Cardston (ARC) plants 144 hr after release (Figures 4-2, 4-3 and 4-4, summarized in Figure 4-5). At this time, the lower confidence limit of the slope of Iwao's patchiness regression overlapped a value of 1, that indicative of Poisson distribution.

The lower confidence limit of the intercept of functions associated with *A. nigriscutis* on plants from each sample, at all time intervals was also less than zero. This indicates that aggregation sizes of greater than one beetle could not be determined with any reasonable certainty on any plants at any time interval. There were no significant differences in slopes or intercepts of functions

associated with Iwao's Patchiness regression for *A. nigriscutis* aggregations on Cardston (ARC), Fort Macleod or Ponoka plants at 4, 24 or 144 hr after release.

Numbers of *A. nigriscutis* per plot at each time interval were examined. There were no block effects ($p=0.8490$), no effect of sample ($p=0.7251$) and no interaction of time and sample ($p=0.1731$). A significant time effect was apparent ($p=0.0001$). *A. nigriscutis* numbers diminished significantly on all plants from 0 to 4 hr and again from 24 to 144 hr after release (Table 4-7).

The numbers of immigrant *A. nigriscutis* per plot were also examined. There was no exchange of beetles between plots until 24 hr after release. There were no significant block effects or differences in numbers of immigrant *A. nigriscutis* per plot ($p=0.1320$ and $p=0.2154$, respectively). The numbers of immigrants, as a proportion of the total numbers of beetles per plot were also compared. There were no significant block effects or differences in the proportions of immigrant beetles per plot ($p=0.3526$ and $p=0.1245$, respectively).

After initial release of *A. nigriscutis* on field plots, there were no significant differences in the levels of aggregation, the numbers of beetles or the proportions of immigrant beetles. *A. nigriscutis* dispersed from initial points of release and established new, ephemeral groups on nearby leafy spurge plants within each plot (Figure 4-6). The distribution of *A. nigriscutis* on Cardston (ARC) plants 144 hr after release, though not significantly less aggregated than that of those on plants from the other sites at this time, was also not significantly different from a Poisson distribution. Whether these beetles were randomly

distributed or distributed in clumps could not be accurately determined at this time interval.

Discussion

No significant differences in responses of *A. nigriscutis* to undamaged leafy spurge plants from these ten samples were observed in laboratory olfactometer tests. Either volatiles from these undamaged plants do not effect close proximity attraction of *A. nigriscutis* to potential host plants or they were not present in concentrations high enough to influence beetle responses.

Although there were no significant differences among the overall responses of *A. nigriscutis* to mechanically damaged Cardston (ARC), Fort Macleod and Ponoka leafy spurge plants, responses of these beetles to increasing damage varied significantly by sample. This apparent contradiction is resolved by examining responses of *A. nigriscutis* to plants from each sample. *A. nigriscutis* responded to mechanically damaged Fort Macleod plants only. They were more attracted to 8 and 16 damaged leaves. That *A. nigriscutis* responded to these mechanically damaged plants alone indicates that their attractiveness to *A. nigriscutis* is influenced by volatiles present within plant tissues that are released by mechanical damage. It also demonstrates differences relevant to *A. nigriscutis* between Fort Macleod plants and the other leafy spurge samples.

Variability among North American leafy spurge populations has been demonstrated for leaf surface compounds (Manners and Davis 1984) and latex constituents (Holden and Mahlberg 1992). Results of this test indicate that this variability includes volatile compounds that are attractive to *A. nigriscutis*. This variability also includes the presence or absence of compounds that influence the attractiveness of leafy spurge plants to *A. nigriscutis*. Differences among *A.*

nigriscutis responses to mechanically damaged plants despite environmental consistencies, common greenhouse conditions and growth media, suggest genetic variability consistent with that demonstrated by Rowe et al. (1997) among North American leafy spurge populations.

More pronounced differences among these plants were demonstrated when responses of *A. nigriscutis* to conspecifics feeding upon them were examined. *A. nigriscutis* feeding upon Ponoka plants were most attractive to conspecifics. Those feeding upon Fort Macleod plants were less, though not significantly less attractive. *A. nigriscutis* feeding upon Cardston (ARC) plants were significantly less attractive than on either Ponoka or Fort Macleod leafy spurge plants.

There were no differences among the responses of *A. nigriscutis* to 20 conspecifics upon any of these plants. This density was determined to be the most attractive to *A. nigriscutis* upon Cardston (ARC) and Ponoka plants and significantly more attractive than uninfested Fort Macleod plants. That a 'suitable resource location and colonisation signal' consistent with that produced by *Prostephanus truncatus* to attract conspecifics to an appropriate food source (Fadamiro and Wyatt 1996, Fadamiro et al. 1996) is produced by *A. nigriscutis* is likely. However, differences among these plants were not great enough to influence production of this signal.

A. nigriscutis fed upon all of the leafy spurge samples that were tested including Cardston (ARC), the least palatable plant tested in feeding trials (Chapter 2). As *A. nigriscutis* has been reared upon this plant (Alec McClay *pers. comm.*), it is a suitable host for these beetles. The lack of significant differences

in the numbers of eggs laid on or near any of the collections tested in feeding trials supports this conclusion. Although there are differences among these plants, they are all likely acceptable hosts and production of aggregation pheromone is the same on all of these plants at this density.

Differences in *A. nigriscutis* responses occurred at higher densities. Beyond 20 *A. nigriscutis* per plant there was a shift of attractiveness to repellence upon Cardston (ARC) plants, a reduction, then an increase and second shift to repellence upon Ponoka plants and sustained attraction until 80 conspecifics per plant and then a shift to repellence upon Fort Macleod plants.

In the case of Fort Macleod plants, sustained attraction is likely due to the attractive effects of plant volatiles and possibly an interaction of volatiles and aggregation pheromone like that demonstrated for members of the Scarabaeidae, Nitidulidae and Curculionidae (Yarden and Shani 1994, Dowd and Bartelt 1991, Weissling et al., 1993). Fort Macleod was the only plant in these tests that produced or released attractive volatiles in response to mechanical damage.

The lack of significant increase in the attractiveness of beetles at low densities on Fort Macleod plants relative to the other leafy spurge samples is likely due to the small amounts of volatiles released or produced by this relatively small amount of feeding damage. As levels of feeding damage increase, greater amounts of attractive plant volatiles are released or produced and *A. nigriscutis* response increases, thus the greatest response at 60 beetles per plant.

As *A. nigriscutis* did not respond to mechanically damaged Cardston (ARC) and Ponoka plants, differences between responses beetles to conspecifics

on these plants at higher densities are not due to volatiles released by mechanical damage. In a previous study of responses of parasitoids to different methods of herbivore feeding, Turlings et al. (1998) found that plant volatile emissions vary with the type of damage. Compounds released from or produced by Ponoka plants in response to *A. nigriscutis* feeding may be emitted in amounts insufficient to influence responses of these beetles until a large amount of feeding damage has been inflicted, thus no effect until higher densities.

The issue of compounds associated strictly with feeding damage was addressed in Chapter 3. Feeding damage by *A. lacertosa* was considered analogous to that of *A. nigriscutis* but did not result in a significantly different response from *A. nigriscutis* than mechanical damage. Cardston (ARC), not Ponoka plants were tested. Analogous conclusions regarding Ponoka plants require that responses of *A. nigriscutis* to *A. lacertosa* on these plants be assessed. Ponoka plants may produce a novel volatile compound(s).

The compound(s) responsible for an increased response at 80 beetles per plant may be attractive plant volatiles or a pheromone precursor. Production of components of *Ips paraconfusus* (Coleoptera: Scolytidae) aggregation pheromone requires exposure to vapours of the host tree monoterpene, myrcene (Hughes 1974). A compound with a similar role may not be released or produced by Ponoka leafy spurge until these beetles inflict a sufficient level of feeding damage.

This compound(s) may also be a plant-produced pheromone constituent. Females of the European elm bark beetle, *Scolytus multistriatus* (Scolytidae)

produce an aggregation pheromone. This pheromone consists of 4-methyl-3-hepanol and (-)- α -multistriatin, both produced by *S. multistriatus* and (-)- α -cubebene, produced by the host tree (Gore et al. 1977, cited from Lanier 1983). Lanier (1983, in Ahmad 1983) suggests that potential host trees that do not produce (-)- α -cubebene when attacked do not contribute to the aggregation pheromone and are not extensively colonized. Which of these, attractive plant volatile, pheromone precursor or pheromone constituent is the most likely to have influenced the attractiveness of *A. nigriscutis* upon Ponoka plants could not be determined with these tests.

Volatile plant emissions contributed to responses of *A. nigriscutis* to conspecifics upon Fort Macleod plants. Another factor contributed to them upon Ponoka plants. Both maintained attractiveness over a larger range of densities than upon Cardston (ARC) plants. Sustained attractiveness may have implications for the rate of *A. nigriscutis* recruitment to conspecifics metapopulations. Due to the combined effects of aggregation pheromone and the sustained attractive influence of plant volatiles, *A. nigriscutis* metapopulation growth should occur at a faster rate and overall responses of conspecifics should be greater upon Fort Macleod than upon Cardston (ARC) plants. As increased attractiveness of Ponoka plants at higher densities was evident, rates of recruitment upon these plants may be similar to Fort Macleod and greater than Cardston (ARC) plants.

A. nigriscutis demonstrated preferences to conspecifics upon these plants similar to those demonstrated in feeding trials in Chapter 2. Ponoka plants were

preferred, followed by Fort Macleod plants. Responses of *A. nigriscutis* to conspecifics upon both of these were significantly greater than those to conspecifics upon Cardston (ARC) plants. That consistent differences were apparent in both the feeding trial and in olfactometer tests supports arguments for distinct biotypes of leafy spurge to be recognized. A biotype is defined as a classification of organism that is morphologically similar to but physiologically different from other members of the species (Arneson 2001).

Despite differences in the attractiveness of *A. nigriscutis* upon these plants to conspecifics, a repellent effect occurred at approximately the same densities on all three collections. Repellent cues, produced by *A. nigriscutis* in response to excessively high conspecific densities take precedence and counter the attractive effects of both volatile plant emissions and attractive cues from these beetles. Production of repellent cues is determined by *A. nigriscutis* density and varied little by leafy spurge collection.

Findings of the field test indicate that differences among these plants did not influence beetle distributions over the time period tested. There were no significant differences in the level of aggregation among groups of *A. nigriscutis* upon these plants at any of the monitoring time intervals, nor were there significant differences among intercepts of these functions at any monitoring time intervals. Differences among total numbers of *A. nigriscutis* or proportions of immigrant *A. nigriscutis* on these plants were also insignificant.

Reductions in *A. nigriscutis* numbers over each monitoring interval may have been partially due to dispersal of these beetles from the plots. Groups of 750

were released upon each plot. These densities were considerably higher than the 120 *A. nigriscutis* per plant found to be repellent in olfactometer tests. Repellent cues produced by such a large number of *A. nigriscutis* may have caused rapid dispersal from these plots shortly after release. These cues would have also deterred exiled *A. nigriscutis* from settling upon neighbouring plots. There was no exchange of beetles between plots until 24 hr after release. Large numbers of ants, spiders and other potential *A. nigriscutis* predators were present on the site and may too have reduced their numbers.

The results of Chapter 2 indicated no correlation between feeding preferences of *A. nigriscutis* with establishment of these beetles on collection sites. There was, however, a correlation between the variability of leafy spurge plants at a site and establishment of *A. nigriscutis* (Chapter 2). Responses of *A. nigriscutis* to differences in attractiveness of conspecifics feeding upon these plants differed significantly but these differences did not influence the distributions of *A. nigriscutis* in this field plot aggregation study. Establishment apparently depends less on the attractiveness of host plants than upon their variability. As each plot in the field trials was a monoculture of plants from each collection, differences in palatability, effects on the production of aggregation pheromone or the attractiveness of volatiles of plants within these plots would have been exerted equally by all plants within each plot and not influenced beetle distribution.

Host discrimination in a heterogeneous patch, however, may promote aggregation behaviour. Aggregation behaviour has been implicated as a factor

that contributes to the success of some biological control agents (Beddington et al. 1978, cited from Begon et al. 1990). An increased level of aggregation in response to a more attractive aggregation pheromone, attractive host plant volatiles or an interaction of these substances may result in an increase in the benefits associated with this distribution: mate location, predator avoidance and an increased ability to overcome host defenses. It may also help *A. nigriscutis* to avoid Allee effects, lower than expected population growth due to an inability of these beetles to find mates, overcome host defenses or avoid predators at low densities (Allee 1931, cited in Begon et al., 1990). Without the influence of multiple host biotypes contributing to a clumped distribution, *A. nigriscutis* may be more randomly distributed and subject to such an effect. Effects of within-site variability on *A. nigriscutis* distribution should be examined.

Tables

<i>SNK Grouping</i>	<i>Mean of $\sqrt{(x+0.5)}$ transformed responses</i>	<i>n</i>	<i>Numbers of Damaged Leaves</i>
A	5.01	40	16
A	4.59	40	8
B	3.58	40	0
B	3.51	40	4
B	3.14	40	2

Table 4-1. 1999 Olfactometer tests. Means separation, by Student-Newman-Keul’s test, of the $\sqrt{(x+0.5)}$ transformed responses of beetles to Fort Macleod plants with increasing levels of mechanical damage. There were no significant differences between like-lettered groups. *n* indicates the number of beetles tested.

SNK Grouping	Mean of $\sqrt{(x+0.5)}$ transformed responses	n	Beetles per plant
A	5.20	40	20
AB	4.98	40	40
AB	4.95	40	80
B	4.39	40	60
B	4.07	40	0
C	3.31	40	100
C	3.29	40	120

Table 4-2. 1999 Olfactometer tests. Means separation, by Student-Newman-Keul's test, of the $\sqrt{(x+0.5)}$ transformed responses of *A. nigriscutis* to groups of 0, 20, 40, 60, 80, 100 and 120 conspecifics on Ponoka plants by Student-Newman-Keul's test. There were no significant differences between like-lettered groups. *A. nigriscutis* were significantly more attracted to groups of 20 conspecifics than to undamaged plants and repelled by groups of 100.

SNK Grouping	Mean of $\sqrt{(x+0.5)}$ transformed responses	n	Beetles per plant
A	5.15	40	60
AB	4.85	40	20
BC	4.38	40	80
BC	4.36	40	40
CD	4.07	40	100
D	3.58	40	0
E	2.76	40	120

Table 4-3. 1999 Olfactometer tests. Means separation, by Student-Newman-Keul's test, of the $\sqrt{(x+0.5)}$ transformed responses of *A. nigriscutis* to groups of 0, 20, 40, 60, 80, 100 and 120 conspecifics on Fort Macleod plants by Student-Newman-Keul's test. There were no significant differences between like-lettered groups. *A. nigriscutis* remained significantly more attractive to conspecifics than undamaged plants until densities of 80 beetles per plant.

SNK Grouping	Normalized mean	n	Beetles
A	5.21	40	20
B	4.28	40	40
BC	4.09	40	60
BC	3.89	40	0
CD	3.42	40	100
CD	3.37	40	80
D	2.78	40	120

Table 4-4. 1999 Olfactometer tests. Means separation, by Student-Newman-Keul's test, of the $\sqrt{(x+0.5)}$ transformed responses of *A. nigriscutis* to groups of 0, 20, 40, 60, 80, 100 and 120 conspecifics on Cardston (ARC) plants by Student-Newman-Keul's test. There were no significant differences between like-lettered groups. *A. nigriscutis* were significantly more attracted to groups of 20 conspecifics than any other sized group and repelled by groups of 120.

<i>SNK Grouping</i>	<i>Mean of $\sqrt{(x+0.5)}$ transformed responses</i>	<i>n</i>	<i>Collection site</i>
A	4.37	280	Ponoka
A	4.18	280	Fort Macleod
B	3.86	280	Cardston (ARC)

Table 4-5. 1999 Olfactometer tests. Means separation, by Student-Newman-Keul's test, of the $\sqrt{(x+0.5)}$ transformed responses of *A. nigriscutis* to conspecifics feeding upon plants from the Ponoka, Fort Macleod and Cardston sites. There were no significant differences between like-lettered groups. Conspecifics feeding upon Ponoka and Fort Macleod plants were more attractive than those upon Cardston (ARC) plants.

<i>Mechanically Damaged Plants SNK Grouping</i>	<i>Conspecifics Feeding upon Plants SNK Grouping</i>	<i>Feeding Trials SNK Grouping (artificial grouping)</i>	<i>Collection Site</i>
A	A	A	Ponoka
A	A	A	Fort Macleod
A	B	B	Cardston (ARC)

Table 4-6. Comparisons of responses of *A. nigriscutis* to mechanically damaged plants from Ponoka, Fort Macleod and Cardston (ARC) collection sites, overall responses of these beetles to groups of 20, 40, 60, 80, 100 and 120 conspecifics feeding upon these plants and feeding preferences of *A. nigriscutis* to these plants as determined in the feeding trials of Chapter 2. The Student-Newman-Keul's grouping associated with feeding trials is artificial. Although it reflects differences demonstrated among these plants in this test, it does not incorporate all of the plants that were tested. Although there were no significant differences in the attractiveness of volatiles from mechanically damaged plants, the same relative preferences as demonstrated in both feeding trials and responses of *A. nigriscutis* to conspecifics feeding upon these plants were evident. Ponoka plants were most preferred; Cardston (ARC) plants were least.

<i>SNK Grouping</i>	<i>Mean of $\sqrt{(x+0.5)}$ transformed responses</i>	<i>n</i>	<i>Hours after release</i>
A	750.0	4	0
B	137.0	4	4
B	113.7	4	24
C	84.2	4	144

Table 4-7. 1999 Field Plot Aggregation Study. Means separation, by Student-Newman-Keul's test, of the numbers of *A. nigriscutis* on all plots. There are no significant differences between like-lettered groups. There were significant reductions in the numbers of beetles per plot between the time of release and 4 hr after release and again between 24 and 144 hr after release. These reductions may have been due to dispersal or to predators.

Figures

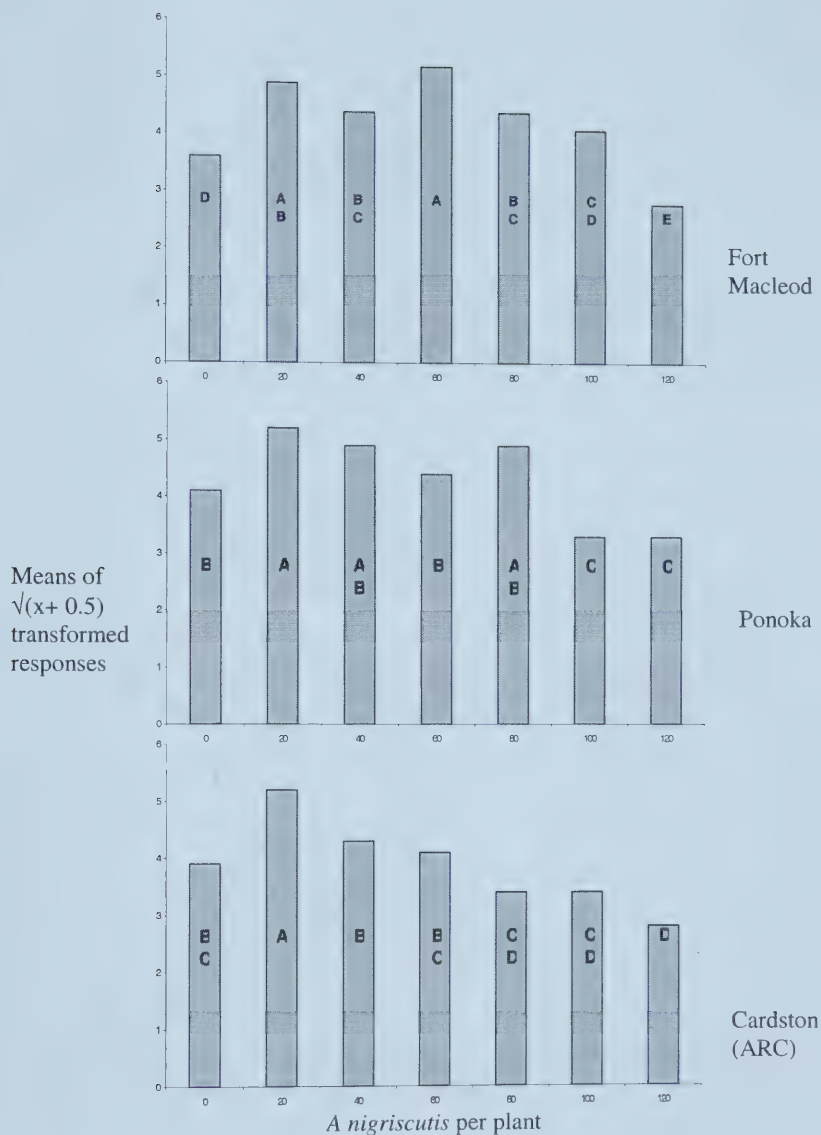


Figure 4-1. Comparison of responses of *A. nigriscutis* to conspecifics feeding upon Fort Macleod, Ponoka and Cardston (ARC) collection plants in olfactometer tests. Each collection is examined separately; Student-Newman-Keul's groupings apply only to other groups on plants from the same collection. Both Ponoka and Cardston (ARC) plants shared relatively similar response trends, with the exception of Ponoka at 80 beetles per plant. Differences between these and responses of *A. nigriscutis* to Fort Macleod Plants are likely due to the combined effects of aggregation pheromone and attractive plant volatiles from Fort Macleod plants. Groups of 120 *A. nigriscutis* were repellent to conspecifics on all three leafy spurge collections.

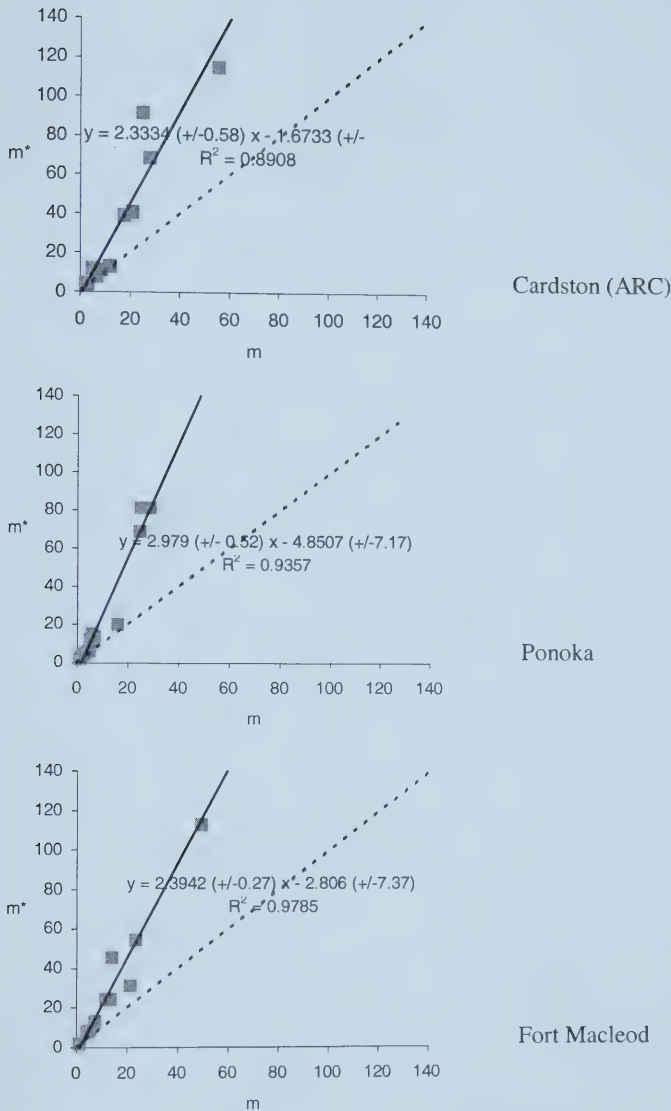


Figure 4-2. Iwao's regression of *A. nigriscutis* distribution on Cardston, Ponoka and Fort Macleod plants at the Ellerslie research farm 4 hr after the release of beetles. The slope of the regression of the Lloyd's mean crowding coefficient (m^*) by the mean numbers of beetles per column of each plot (m) or the 'index of basic contagion' indicates the distribution of beetles. Slopes greater than 1 (indicated by the dotted line) indicate an aggregated distribution. The intercept or 'density contagiousness index' indicates the numbers of beetle per aggregate. Intercept values greater than 0 indicate that the aggregate is composed of multiple insects. The slopes of all functions are greater than 1. There were no significant differences in the functions associated with beetles on Ponoka, Cardston or Fort Macleod plants.

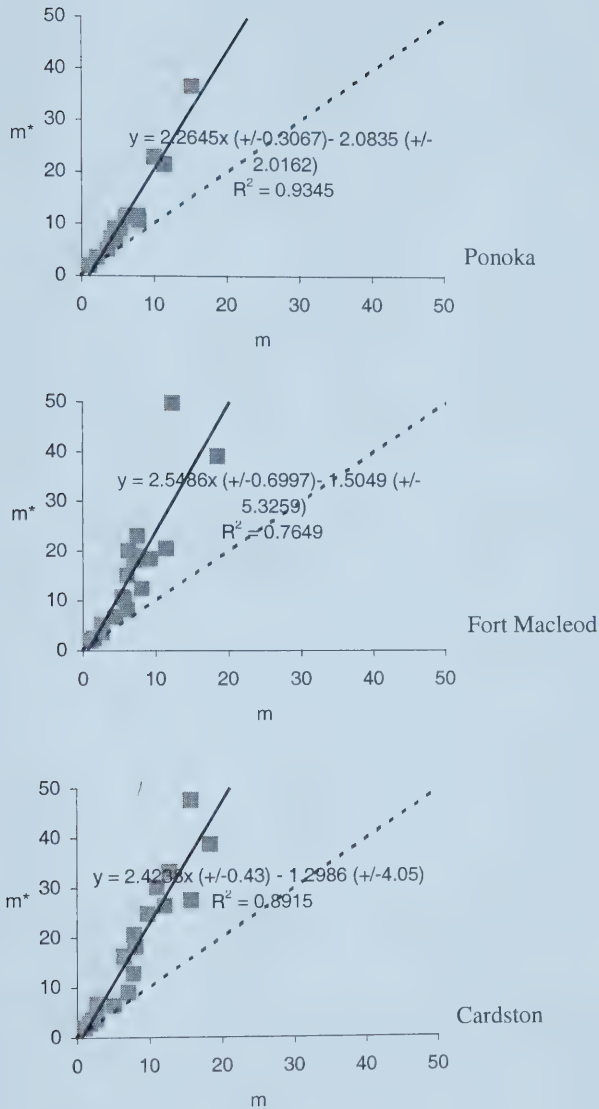


Figure 4-3. Iwao's regression of *A. nigriscutis* distribution on Cardston, Ponoka and Fort Macleod plants at the Ellerslie research farm 24 hr after the release of beetles. The slope of the regression of the Lloyd's mean crowding coefficient (m^*) by the mean numbers of beetles per column of each plot (m) or the 'index of basic contagion' indicates the distribution of beetles. Slopes greater than 1 (indicated by the dotted line) indicate an aggregated distribution. The intercept or 'density contagiousness index' indicates the numbers of beetle per aggregate. Intercept values greater than 0 indicate that the aggregate is composed of multiple insects. The slopes of all functions are greater than 1. There were no significant differences in the functions associated with beetles on Ponoka, Cardston or Fort Macleod plants.

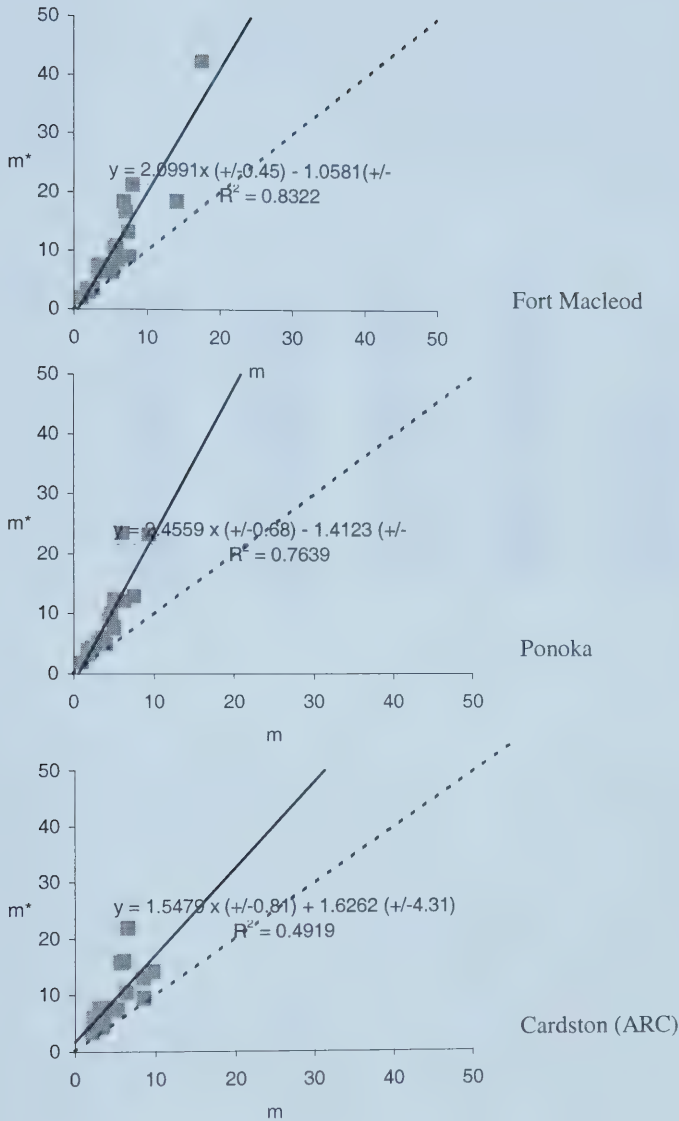


Figure 4-4. Iwao's regression of *A. nigriscutis* distribution on Cardston, Ponoka and Fort Macleod plants at the Ellerslie research farm 144 hr after the release of beetles. The slope of the regression of the Lloyd's mean crowding coefficient (m^*) by the mean numbers of beetles per column of each plot (m) or the 'index of basic contagion' indicates the distribution of beetles. Slopes greater than 1 (indicated by the dotted line) indicate an aggregated distribution. The intercept or 'density contagiousness index' indicates the numbers of beetle per aggregate. Intercept values greater than 0 indicate that the aggregate is composed of multiple insects. The slopes of all functions except that associated with beetles on Cardston plants are significantly greater than 1.

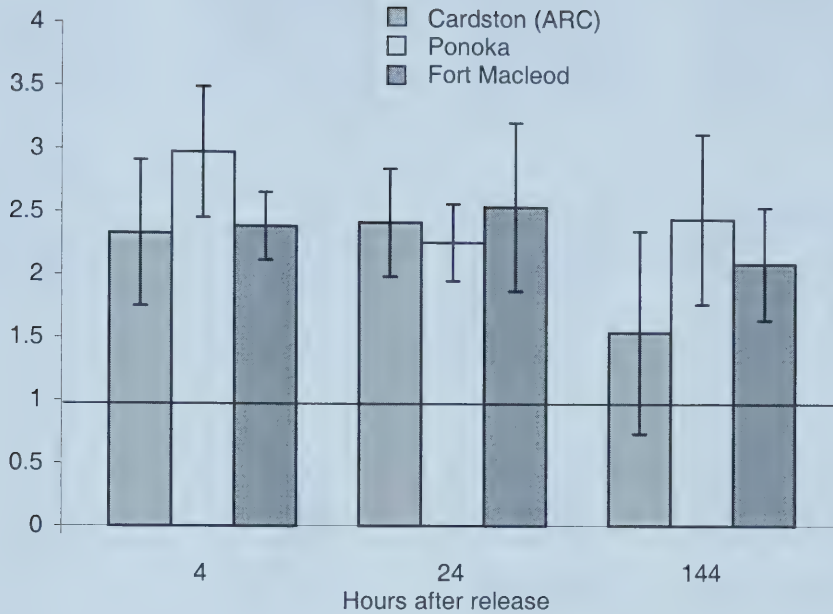


Figure 4-5. Comparison of the 95% confidence intervals of the slopes associated with the regression of Lloyd's mean crowding coefficient by the mean numbers of beetles per column of arrays of Cardston, Ponoka and Fort Macleod plants at three monitoring intervals. The slope of this function indicates the level of aggregation of beetles on plants at these times. Value greater than '1' indicate an aggregated distribution. Values not significantly different than '1' indicate Poisson distribution. There were no significant differences in the levels of aggregation of beetles on these biotypes at any time interval though there were significant differences between that for beetles on Ponoka plants 4 hr after release and on Cardston plants 144 hr after release. The distribution of beetles on Cardston plants at 144 hr after release was not significantly different from the Poisson distribution.

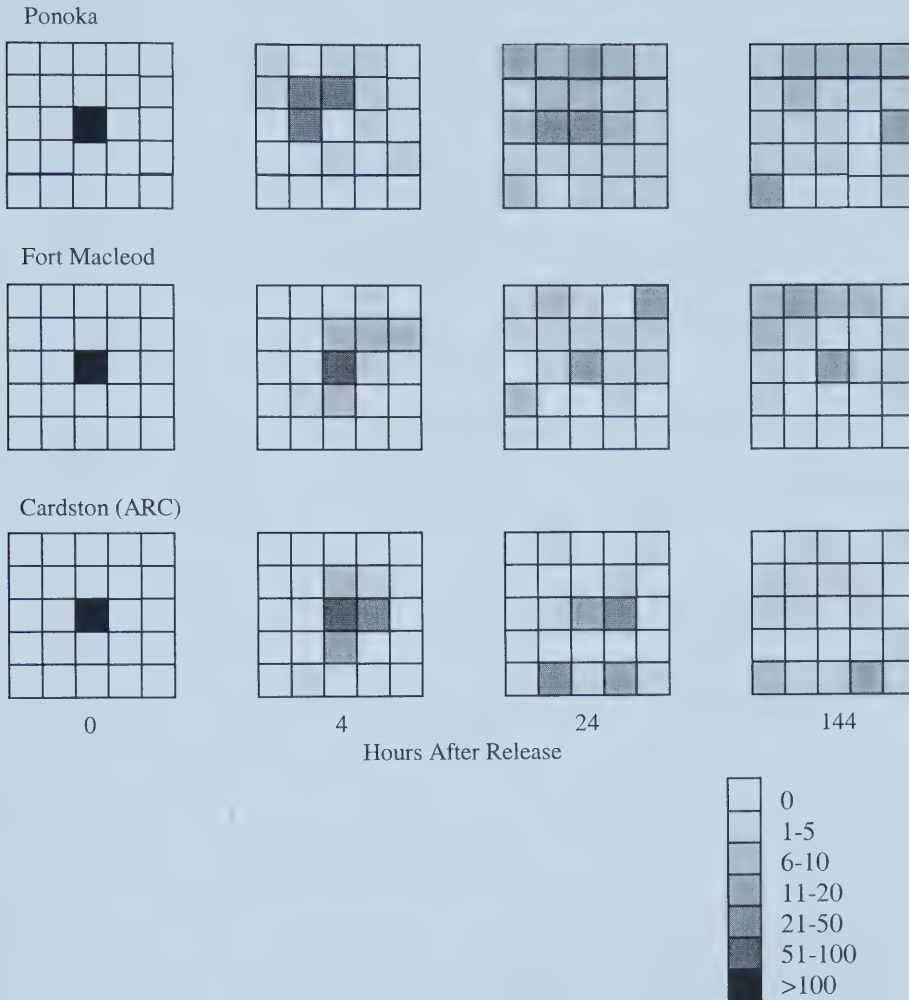


Figure 4-6. Diagrams representing numbers of *A. nigriscutis* per plant on plots with Ponoka, P, Fort Macleod, F and Cardston (ARC), C plants. Each square represents a single plant. Beetles dispersed from the original points of release to form new, ephemeral congregations on plots with these leafy spurge plants.

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Chapter 5

Conclusions

Studies of feeding preferences of *A. nigriscutis* for the leafy spurge samples examined here indicate that there are relevant differences among them. In previous studies of specialist insect herbivores examined as biological control agents of this weed, Harris et al (1985) suggested that latex or toxin chemistry are influential in host-choices and suitability of spurge species. Holden and Mahlberg (1992) documented differences among latex constituents in North American spurge while Manners and Davis (1984) demonstrated differences in leaf surface wax compounds associated with North American spurge and between these and European leafy spurge sources. These differences included variations in the concentrations of the leaf wax triterpenoids, α -amyirin and β -amyirin, potentially anti-feedant substances found to be present in higher concentrations in azalea varieties resistant to the azalea lace bug *Stephanitis pyrioides* (Heteroptera: Tingidae) (Balsdon et al 1995) and crucifers resistant to diamondback moth larvae, *Plutella xylostella* (Lepidoptera: Plutellidae) (Eigenbrode et al 1991) than in susceptible varieties. Leaf and latex constituents or other compounds may have influenced *A. nigriscutis* feeding preferences in these feeding trials.

Causes for differences among *A. nigriscutis* feeding preferences could not be assessed with these tests but as these differences were demonstrated despite controlled environmental conditions, common greenhouse conditions and growth medium, genetic variability as demonstrated by Rowe et al (1997) among other North American leafy spurge collections seem likely. Consistent differences in feeding preferences maintained after a year in a greenhouse support this

conclusion and suggest that distinct biotypes of leafy spurge should be recognized. A biotype is defined as a classification of organism that is morphologically similar to but physiologically different from other members of the species (Arneson 2001). Differences in palatability suggest different physiologies.

Olfactometer assessments of the responses of *A. nigriscutis* to conspecifics feeding upon some of these plants indicated the same preferences. Conspecifics feeding upon Ponoka plants were most attractive and those upon Cardston (ARC) plants were least. Conspecifics feeding upon Fort Macleod plants were found to be intermediately attractive but significantly more so than those upon Cardston (ARC) plants.

Differences in responses of *A. nigriscutis* to conspecifics upon Ponoka and Cardston (ARC) plants may be due to the production or release of a novel attractive plant volatile in response to beetle feeding upon Ponoka plants or differences in pheromone production by conspecifics upon these collections. These differences may indicate variability in a plant-produced pheromone constituent, as demonstrated for *Scolytus multistriatus* (Scolytidae) (Gore et al 1977, cited from Lanier 1983), or a pheromone precursor, as demonstrated for *Ips paraconfusus* (Coleoptera: Scolytidae) (Hughes 1974). Differences were only apparent at relatively high beetle densities. Compounds may only be released from or produced by Ponoka plants in response to *A. nigriscutis* feeding and may be emitted in amounts insufficient to influence responses of these beetles until a

large amount of feeding damage has been inflicted, thus no effect until higher densities.

At a relatively low density, 20 beetles per plant, there were no significant differences among responses of beetles to conspecifics upon these plants. This density was the most attractive upon Ponoka and Cardston (ARC) plants and significantly more attractive than undamaged Fort Macleod plants. On Fort Macleod plants, this group size was not significantly less attractive than the most attractive density. A 'suitable resource location and colonisation signal' consistent with that produced by *Prostephanus truncatus* to attract conspecifics to an appropriate food source (Fadamiro and Wyatt 1996, Fadamiro et al 1996) produced by *A. nigriscutis* is likely. However, differences among these leafy spurge collections were not great enough to influence production of this signal at this density.

As *A. nigriscutis* fed and oviposited upon all of the leafy spurge samples tested and has been reared upon the least palatable of these, all are probably acceptable hosts. However, Gassman (1996) demonstrated differences in *A. nigriscutis* larval survival among spurge species. Feeding preferences may reflect differences in the quality of these leafy spurge collections as hosts to *A. nigriscutis*, even though all can support these beetles. Differences in feeding preferences may be a response to variable latex or toxin compounds. If these substances influence or indicate the host quality of leafy spurges for other insects, as suggested by Harris et al 1985, they may also influence it for *A. nigriscutis* and be detectable by these beetles. For the crucifer feeding *Phyllotreta cruciferae*

(Chrysomelidae: Alticinae) survival is lower on crambe (*Crambe abbyssinica*) than on rape (*Brassica napus*) and they feed less upon it (Peng et al 1992). *A. nigriscutis* may also respond to leaf surface substances like potentially anti-feedant α -amyrin and β -amyrin. The influence of these compounds on *A. nigriscutis* remains untested.

Variable host volatiles and/or production of aggregation pheromone in response to an as yet unknown host plant factor may have also contributed to differences in *A. nigriscutis* feeding responses demonstrated in Chapter 2. *A. nigriscutis* were presented with a number of potential hosts and some of these leafy spurge plants differed in a characteristic(s) that influenced feeding preferences. As *A. nigriscutis* demonstrated the same preferences for leafy spurge collections in olfactometer tests of responses to conspecifics feeding upon them and in feeding trials, both the results of feeding trials and the attractiveness of conspecifics upon these plants are likely influenced by the same factor(s).

Assuming that plant volatiles from undamaged plants did not influence host choices before these beetles made physical contact with leafy spurge plants, distribution of *A. nigriscutis* shortly after introduction to feeding trials was likely random. As these beetles did not respond to undamaged plants in olfactometer tests and dispersed rapidly throughout the feeding trial arenas and as almost all plants in these trials suffered some damage, this is probably the case. In feeding trials, random encounters with potential host plants and a plant characteristic(s) that influences both feeding preferences and the attractiveness of conspecifics upon these plants, suggest the following scenario.

A male randomly encounters a leafy spurge plant in a feeding trial arena and responds to leaf surface compounds, plant latex or some other factor(s). Some leafy spurge biotypes in the arena may possess traits that impart anti-xenosis or antibiosis resistance to these beetles. Some may also possess characteristics that act as feeding stimulants. As the cucurbit specialist *Diabrotica* spp. respond to the amounts of cucurbitacins B and E in their host plants (Metcalf et al 1979) it is reasonable to assume that spurge specialist *A. nigriscutis* also responds to compounds with similar roles in *Euphorbia* spp..

These feeding trials were choice tests with a number of leafy spurge biotypes in close proximity. *A. nigriscutis* were not obliged to settle and feed upon one plant as they were in olfactometer tests. Male beetles may sample until they find a plant that stimulates a host-type response. Although the factors that influence these choices remain unclear, it is apparent that, as beetles sampled most plants in these tests, feeding is required for *A. nigriscutis* to assess potential hosts. Once a suitable plant is located, aggregation pheromone is produced. Aggregation pheromone attracts conspecifics, including other males that, as a consequence of contact with the plant, are stimulated to produce aggregation pheromone. More conspecifics are attracted, produce more aggregation pheromone and attract more conspecifics. This feedback system has the potential to rapidly increase the attractiveness of *A. nigriscutis* congregations and would be more dramatic upon a plant that sustains this attraction until greater densities.

Significant row and column effects were apparent in some feeding trials. By surface plotting trials that demonstrated these effects, plants in the area

immediately around preferred leafy spurge plants were found to sustain greater damage than other clones from these leafy spurge samples that were adjacent less attractive plants. As larger amounts of foliar damage can be attributed to greater numbers of *A. nigriscutis*, beetle aggregations were centred upon the most palatable plants. Beetles on the periphery of aggregations fed upon plants adjacent to this plant regardless of these plants' palatability. Apparently, in choice situations feeding preferences dictate initiation of the feedback loop but once started they become secondary to the influence of aggregation pheromone(s). Attractive plant volatiles or plant traits that effect pheromone production may have influenced results of feeding trials by attracting more *A. nigriscutis* to larger congregations and contributing to a feedback effect.

A potential interaction of plant volatiles and *A. nigriscutis* pheromones, like that demonstrated for members of Scolytidae, Scarabaeidae, Nitidulidae and Curculionidae (Wood 1982, Yarden and Shani 1994, Dowd and Bartelt 1991, Lin et al 1992, Weissling et al. 1993), might also influence *A. nigriscutis* behaviour. Distinguishing additive from synergist effects of beetle and plant volatiles without isolating these compounds is difficult. However, according to Lance (1983 cited in Ahmad 1983) synergistic plant compounds are generally neutral or slightly repellent alone but very attractive when in combination with other compounds. As volatile emissions from Fort Macleod plants were attractive to *A. nigriscutis*, the mode of interaction of beetle pheromones and these plant volatiles is likely additive. If volatiles were released from or produced by mechanically damaged Ponoka plants, they were not attractive to *A. nigriscutis*. Although this does not

offer clear proof of a synergistic interaction of Ponoka plant volatiles, released or produced in response to large amounts of beetles feeding, and *A. nigriscutis* aggregation pheromone it does support this possibility.

The compounds responsible for an increase in response at 80 beetles per Ponoka plant may also be pheromone precursors or plant-produced pheromone constituents. However, as an aggregation pheromone was already influencing beetle behaviour at densities of 20 per plant, the possibility of a pheromone precursor produced or released in response to heavy beetle feeding is somewhat diminished.

Variable plant volatiles, aggregation pheromones or variable pheromone constituents do not influence *A. nigriscutis* dispersal. Large groups of *A. nigriscutis* were repellent to conspecifics at approximately the same high densities on all plant collections tested in the olfactometer despite the attractive influence of aggregation pheromone, plant volatiles and/or pheromone constituents. These cues are of secondary importance to dispersal cues at this density but they do influence the attractiveness of *A. nigriscutis* to conspecifics feeding upon leafy spurge plants before this density is reached.

Groups of *A. nigriscutis* were more attractive upon Fort Macleod plants than undamaged plants from this sample until densities of 80 beetles per plant. Cardston (ARC) plants were attractive, relative to undamaged plants from this sample, only at densities of 20 beetles per plant. Ponoka plants were attractive at 20, 40 and 80 beetles per plant. Plant volatiles or an interaction of plant compounds and *A. nigriscutis* aggregation pheromone prolonged an attractive

effect of these beetles on Fort Macleod and Ponoka plants. These plants may suffer more adult beetle feeding and as a consequence of greater beetle numbers, more oviposition than leafy spurges that lack these attractive cues. Although they did suffer more feeding, no such increase in oviposition was evident in feeding trials.

A. nigriscutis completely defoliated some plants in feeding trials. It is important to consider the size of these plants. All were approximately 5 cm in height, considerably smaller than those typically infested by large numbers of *A. nigriscutis*. However, if *A. nigriscutis* dispersal is intended to coincide with densities that threaten resource integrity, why didn't these beetles disperse when these plants had obviously suffered more damage that they could sustain without perishing and were in some cases completely destroyed? *A. nigriscutis* dispersal may depend less upon host plant size and integrity than on conspecific density. These beetles were significantly more attracted to groups of 20 conspecifics than to groups of 120 even without plants in olfactometer tests. Density was also the prevalent factor determining the onset of repellence in olfactometer tests of the three leafy spurge collections tested and occurred at roughly the same density on all plants tested.

Groups of one thousand *A. nigriscutis* were introduced to each feeding trial in 1999. As the most attractive plants in these trials were represented ten times and that there was considerable feeding upon other plants, beetles likely never massed upon individual plants in numbers large enough to initiate mass production of repellent cues. There were also other congregations within each

arena. These could accommodate superfluous beetles repelled from overly dense congregations. As there were no significant differences in comparisons of the feeding preferences of *A. nigriscutis* to the same leafy spurge collections in 1998 and 1999 despite the higher beetle numbers involved in 1999 tests, if a repellent effect was evident it did not influence the results.

An important conclusion drawn from feeding trials, aside that there are significant between- and within-site differences in feeding preferences of *A. nigriscutis* to leafy spurge plants, is that within-site variability was correlated to the size of beetle populations upon a site but overall palatability of plants from each site was not. Although there may be differences in the suitability of these leafy spurge samples, based upon feeding preferences, none of the plants tested could be eliminated as suitable hosts for *A. nigriscutis*. These beetles fed upon all of the leafy spurge collections tested and have been successfully reared upon the plants collected in 1991 from the Cardston site (Cardston (ARC) plants) (Alec McClay *pers. comm.*). As these were the least palatable of the plants tested, the rest of the test plants are also likely to be suitable hosts for *A. nigriscutis*. The lack of significant differences in the numbers of eggs laid on or near any of the collections tested in feeding trials supports this conclusion.

Feeding preferences in these feeding trials and attractiveness of groups of *A. nigriscutis* feeding upon these plants are apparently influenced by the same factor(s). There were within-site differences in feeding preferences. Within-site differences in the attractiveness of *A. nigriscutis* upon these plants also likely

exist. These differences would influence congregation behaviour. The metapopulation maintenance model proposed in Chapter 3 requires elaboration.

A male *A. nigriscutis* is the initial coloniser in a mixed biotype stand of leafy spurge. Factors that draw him to host plants remain unclear but initial choices may be random. If this plant possesses anti-feedant qualities, a trait that imparts anti-xenosis resistance, he moves to a nearby plant. Should this plant provide the appropriate host type stimuli, perhaps feeding stimuli, he produces an aggregation pheromone to draw conspecifics. Attractive plant volatiles are also produced in response to or released by damage associated with his feeding, should this plant possess this trait. As little of this compound is produced by his feeding damage, these volatiles do not significantly increase the attractiveness of this coloniser to nearby conspecifics. Conspecifics are initially drawn to his aggregation pheromone. Assuming that conspecifics are within the range of influence of these cues, they are drawn to the location of the colonizing male, feed and produce aggregation pheromone. They also cause damage to the plant and promote the release of more attractive volatiles. Plant volatiles and aggregation pheromone contribute to a feedback effect and draw more conspecifics.

There may also be other colonisers feeding upon nearby leafy spurge. These males would be in competition for females. Should one of them have colonized a plant that promotes the production of more attractive cues and/or releases or produces more attractive volatiles, more conspecifics would be drawn to his plant. A male's reproductive success may depend upon the attractiveness of the metapopulation he founds or patronises.

As metapopulations grow beyond a threshold density, repellent cues are produced and superfluous *A. nigriscutis* disperse. Dispersal cues are produced on all plants at approximately the same *A. nigriscutis* density. However, plants that promote production of more attractive pheromones or emit more attractive volatiles may reach dispersal densities sooner than those that promote less attractive cues. More rapid cycling of aggregation and dispersal may allow more total *A. nigriscutis* to feed upon and lay eggs at the base of these plants. More eggs result in more larvae and more associated damage to the root system of this plant. More attractive plants on a variable site may be suppressed more quickly than less attractive ones.

Attraction of *A. nigriscutis* to conspecific cues may have consequences for how sites are monitored and may allow manipulation of their populations. The most popular *A. nigriscutis* monitoring technique involves sampling the area immediately around release sites with sweep nets. There is a possibility that small groups of highly aggregated or sparsely distributed beetles may be overlooked. Also, as females are likely preferentially attracted to congregations, samples might be more female-biased than entire populations. Pheromone traps, assuming that precise differences in responses of males and females to male-produced aggregation cues and the effective range of these cues are established, may be a way to more accurately sample *A. nigriscutis* populations on release sites. Pheromone-baited beacons may also be a way to encourage the spread of beetles to particular points of a weed stand, reducing potential Allee effects, or drawing *A. nigriscutis* into an area that might otherwise be neglected.

Although within-site variability of host plants may help *A. nigriscutis* to avoid Allee effects (Allee 1931, cited in Begon et al 1990) it does not guarantee growth of an *A. nigriscutis* population. *A. nigriscutis* populations are subject to other factors. Soil pH in particular was correlated with *A. nigriscutis* population size on the sites that were examined. Acidic soils, regardless of the factors that contribute to acidity, negatively influence *A. nigriscutis* populations. That Allee effects are avoided and *A. nigriscutis* eggs are laid near plants may not be sufficient to assure development of the next generation. Acidic soils may prove to be an unsuitable environment for subterranean *A. nigriscutis* larvae. Without larval development, the impact of *A. nigriscutis* as a biological control agent may be minimized. These beetles can completely defoliate leafy spurge plants on release sites without significantly affecting these plants. Larval damage to leafy spurge roots causes the bulk of negative effects and results in target weed suppression (Hansen et al 1997). A closer examination of the effects of soil pH, as well as potential interactions of this factor with others, is warranted.

Predators, parasites and pathogens also have the potential to influence biological control agent establishment (Myers 1987). Predators have reduced the success of introduced cactus biocontrol agents at 25% of their release sites and parasites another 7% (Moran and Zimmerman 1984 cited from Barbosa and Schultz 1987). Generalist predators such as ants, that are present on most *A. nigriscutis* release sites, readily adapt to and exploit new resources, such as introduced insects (Myers 1987). Predators may reduce beetle numbers and influence establishment and subsequent suppression of the target weed and may

have greatly reduced *A. nigriscutis* numbers on both the Hardisty and Ellerslie plots. Their influence upon *A. nigriscutis* establishment should also be examined.

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